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PARASITOLOGY

EDITED BY

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VOLUME XIII JUNE, 1921 No. 2

ON THE LIFE-HISTORY OF *HELICOSPORIDIUM PARASITICUM*, N.G., N.SP., A NEW TYPE OF PROTIST PARASITIC IN THE LARVA OF *DASYHELEA OBSCURA* WINN. (DIPTERA, CERATOPOGONIDAE) AND IN SOME OTHER ARTHROPODS.

BY D. KEILIN, Sc.D.

Beit Memorial Research Fellow.

(*From the Quick Laboratory, University of Cambridge.*)

(With Plates IV—VI and 5 Text-figs.)

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I. PRINCIPAL HOST OF *HELICOSPORIDIUM* AND ITS INFECTION.

THE usual host of this new parasite, *Helicosporidium parasiticum*, is the larva of a Ceratopogonid, *Dasyhelea obscura* Winnertz, which lives in the decomposed sap filling the wounds of trees—elm and horse-chestnut. All the material used for this study was derived from the wounds of two trees only: (1) an elm tree standing on the Caius College ground at Newnham, facing Church Rate

Walk, and (2) a horse-chestnut standing between the School of Agriculture and Downing College.

In addition to the new parasite, the larvae of *Dasyhelea* harbour several other parasites, two of which—(1) a gregarine, *Allantocystis dasyhelei* Keilin, and (2) a parasitic yeast *Monosporella unicuspidata* Keilin—have already been dealt with in my previous papers (1920 *a* and *b*).

To find the larvae parasitised with *Helicosporidium*, one proceeds in the following manner: to a small quantity of the semi-fluid exudate collected from the wounds of trees, is added ordinary tap water sufficient to cover the bottom of a wide dish. The mixture is then thoroughly stirred and is left for an hour or more to settle. The numerous larvae of various sizes found at the bottom of the dish are then transferred by means of a pipette to a dish containing clean water and standing on a black or dark coloured surface. On careful examination of the contents of this dish it is noticeable that while the normal *Dasyhelea* larvae present a whitish but translucent appearance, a few individuals are usually seen which are white and opaque. Isolated on a slide and examined under the microscope, all these opaque larvae are found to be parasitised either with *Monosporella*, or with the new parasite *Helicosporidium*, or very exceptionally with both organisms together. As the proportion of larvae parasitised with *Monosporella* is very small, almost all the opaque and milky specimens of *Dasyhelea* larvae are found to be infected with the new parasite.

If we examine under the microscope a parasitised larva, compressed between slide and coverslip, sufficient to prevent movement, we see that its entire body cavity is filled with small round corpuscles, 5 or 6 μ in diameter. These corpuscles occupy all the spaces between the organs of the larva and extend through all the segments including the head.

Being free in the body cavity of the larva, the parasitic corpuscles are always seen circulating or even gushing from one segment to another. This movement is purely passive and is produced either by the contraction of the segments of the host, by the more or less regular movement of its internal organs, or by the rhythmic contractions of the heart and the consequent plasma circulation. These passive movements of *Helicosporidium* are very easily seen in the head of the larva, where the parasites are less densely crowded on account of the restricted spaces between the strongly developed muscles of the mouth-parts. The post-abdominal segments on the contrary are filled to such an extent with the parasitic corpuscles that these form a solid mass occupying the whole cavity of these segments. The posterior portion of the larva becomes thus very turgid, loses its mobility, and becomes very fragile. By pricking such parasitised larva with a fine needle or even by gentle pressure on the coverslip a milky fluid gushes from the wound, and this fluid when examined with the microscope is seen to be a pure suspension of parasitic corpuscles (Pl. V, figs. 1, 2 and 3).

All the larvae which were recognised from their external appearance as being infected, had already arrived at such an advanced stage of infection, that

no stages of the multiplication of the parasite could be detected. To find the earlier stages of infection it was impossible to select the parasitised larvae with the naked eye. For this purpose each larva had to be examined separately under a high magnification, a very long and difficult task, because (1) the larvae are insufficiently transparent owing to their cuticle being lined with a layer of the fat body, whilst (2) the early stages of the parasites are minute and easily confused with droplets of fat or albuminoid corpuscles which often escape from the fat body of the slightly compressed larva.

After selecting living larvae which I suspected to contain the early stages of the parasites, I proceeded to make smears of their bodies, which after fixation and staining, revealed, with very few exceptions, the early stages of this parasite.

In this way I have collected a fairly rich material showing the various stages of the parasite. This material was studied in the form of smears as well as in sections of the larva.

As to technique, I may state that all classical methods of Protozoology were used. I have obtained the best results from the smears fixed in Schaudinn's fluid (with the addition of 1 per cent. acetic acid) and stained in iron-haematoxylin and from sections of the larvae fixed in Carnoy's fluid and stained also in iron-haematoxylin or in haemalum.

II. THE LIFE-HISTORY OF *HELICOSPORIDIUM PARASITICUM*.

1. *Localisation of the parasite.*

All the stages of the parasite are usually found free in the body cavity of the host. In several cases, however, especially when the infection was only recent, the parasites were found either in the fat body or in nerve ganglia. When they attack the fat body, the latter is rapidly destroyed and the parasites, attached to the fat droplets, escape into the body cavity. On the contrary when the nerve ganglia are infected, the infection remains for a long time localised; all the stages are then present simultaneously in the ganglia which become swollen and reduced to the neurilemma. It is interesting to remark that several successive nerve ganglia of the ventral chain may be infected, but the parasites are never found in the nerve commissures.

2. *Early stages and schizogony.*

The youngest stage of the parasite found in the tissue or in the body cavity of the host, is represented by small round corpuscles of 2 or 3μ in diameter (Pl. IV, figs. 1, 2, 3); they are sometimes oval in shape, being then 3μ long and 1·5μ wide. The protoplasm of these corpuscles is homogeneous, being devoid of granulations and vacuoles. The nucleus, in the form of a spherical chromatic granule 0·5μ in diameter is surrounded by a clear zone of protoplasm of 0·75μ in diameter. This clear zone may be the real nucleus, while the chromatic granule is the nucleolus—this, however, could not be proved, as it was im-

possible to detect any nuclear membrane surrounding the clear portion of the protoplasm.

In this stage the parasite grows a little, and then divides into two (Pl. IV, figs. 3, 4, 5); the smallness of the parasite makes it very difficult, if not impossible, to follow in detail the mode of division. In some cases it appeared to me to be an ordinary amitotic division; in other cases on the contrary I could see a fairly clear mitosis. The two cells resulting from the division are of almost equal size and shape (Pl. IV, figs. 6, 7, 8 and 9); they are now more elongated and their protoplasm becomes more basophile. They grow a little, undergo a second division (Pl. IV, figs. 10, 11 and 12) and give rise to a small schizont (or morula) composed of four cells disposed in a tetrahedral manner (Pl. IV, figs. 13 and 14). In a few cases only I have observed all four cells symmetrically arranged quadrantally in one plane (Fig. 15). The schizont composed of four merozoites is usually slightly oval and measures 4μ by 3μ. These schizonts continue their development in two different ways: (1) either by breaking up into four merozoites (Pl. IV, figs. 16 and 17) which being set free, divide in their turn, or (2) by undergoing a third division (Fig. 18) and giving rise to schizonts composed of eight cells or merozoites (Pl. IV, figs. 19, 20). These schizonts, 4μ in diameter, are very basophile, so that it is often difficult to differentiate their nuclei. They undergo no further division, but break up into eight merozoites (Pl. IV, figs. 21 and 22), measuring $1{\cdot}7\mu$–2μ by 1μ which probably divide again in their turn.

I have not yet observed schizonts composed of more than eight cells. This multiplication, which forms an endogenous or schizogonic part of the life-cycle of *Helicosporidium* is very active and always results in the formation of an enormous number of unicellular corpuscles scattered throughout the body cavity of the host or invading its various tissues.

3. *Formation and structure of the spores.*

After a period of very active schizogonic multiplication the parasite passes into the second phase of its life-cycle, namely the formation of spores. The merozoite resulting from the schizogony increases slightly in size, becomes very basophile, and after two successive divisions (Figs. 23, 24 and 25) forms a morula of four cells tetrahedrally disposed. Of these cells, one grows more rapidly than the others and the whole morula completely loses its regular shape (Figs. 26, 27 and 28).

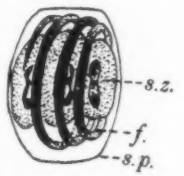
We now arrive at a very short phase in the life-cycle of the parasite, in which the latter undergoes some changes, the nature of which I was unable to follow clearly. However, by a few fragmentary observations and especially by the subsequent development, I think I have succeeded in reconstructing this missing stage, which I shall consider for the present as being hypothetical. Of the four cells which form the morula, one, the fourth, which lies now separately on one side and is much larger than the three other cells, finally

succeeds in surrounding them in the form of a ring. The three surrounded cells change their shape, becoming flattened, in the form of three superposed or parallel discs which occupy the centre of the ring formed by the fourth cell (Figs. 29 and 30). This cell then secretes an external membrane which envelopes the whole group of four cells, thus forming a spore.

We now arrive at a stage in which the spore is most frequently encountered and which is easily recognised. It is the barrel-shaped spore surrounded by a very fine and transparent membrane or sporocyst. Its largest diameter is between 5 and 6μ. When examined end-wise, the spore shows a central circular mass, strongly basophile, and surrounded by a highly refractive ring which fills the space between the central mass and the walls of the sporocyst (Pl. IV, fig. 31).

Examined from the side, the spore shows that the deeply-stained central mass is composed of three superposed discs, parallel to each other and to the flattened surfaces of the barrel-shaped spore (Pl. IV, fig. 32). These central discs are surrounded by a refractive ring which we now see from the side only.

Only by careful differentiation can the vesicular nuclei be detected in the central discoidal cells. These spores now undergo a further transformation: examined *in vivo* they show a spiral refringent band surrounding the three central cells and lining the sporocyst. The more detailed structure of this stage can be seen only in fixed and stained smears or in sections of the infested larva.



Text-fig. 1. Completely formed spore showing the three central cells or sporozoites—*s.z.*; a peripheral spiral filament—*f.*; and the external membrane or sporocyst—*s.p.*.

The protoplasm of the three central cells now loses more and more its basophile property, while in the refringent ring which surrounds these cells a chromatic substance appears which assumes the form of a spiral band with 3–4 turns surrounding the central cells of the spore (Text-fig. 1 and Pl. IV, figs. 33, 34, 35, 36 and 37). Viewed from the polar ends of the spore, the chromatic spiral band appears as a series of superposed chromatic rings surrounding the central cells (Pl. IV, figs. 38–42). During differentiation after staining with iron-haematoxylin, the chromatic spiral still retains a very dark colour, after all the rest of the spore is completely decolorised.

At this stage the nuclei of the central cells are distinct; the nuclei are variable in shape but are usually discoidal, their chromatin forming a peripheral ring which is connected with a central body or karyosome of an irregular form.

The parasite now invades the whole body of its host to such an extent that the latter dies, and as we have seen, the host's tissues are destroyed and replaced by a solid mass composed solely of these spores. For a long time I supposed that these spores represented the final developmental stage of the parasite, namely a resistant form, which, being set free from the dead host, were swallowed by other larvae of *Dasyhelea* which thus became infected.

However, a further study of the parasite showed this supposition to be wrong, for the spores above described were found to undergo further development in the dead body of their host.

4. *Development of the spores subsequent to the death of the host.*

The decomposing sap collected from the wounds of trees often contains dead *Dasyhelea* larvae which, on microscopic examination are found to be completely filled with elongated filaments 60–65μ long, with pointed extremities (Pl. V, figs. 8 and 9). As these filaments show a close resemblance to the acicular spores of yeasts of the genus *Monosporella*, one species of which I have described as being parasitic in the larva of *Dasyhelea*, I was at first under the impression that the elongated filaments were the spores of a similar yeast.

Subsequent observations showed me that such was not the case. These filaments have nothing to do with yeast, but actually belong to a later phase of the development of the spores of *Helicosporidium*. I have been successful in tracing the consecutive steps in the formation of these free filamentous structures from the barrel-shaped spores. If we isolate a parasitised *Dasyhelea* larva in a drop of ordinary tap water or in a small quantity of decomposed sap and leave it to itself it will soon die, being killed by the parasite. On allowing the body of this larva, filled with the barrel-shaped spores, to dry very slowly no noticeable change will occur in the spores; but if the dried body is moistened again, it swells up and a large number of filaments similar to those we have previously mentioned will appear among the barrel-shaped spores. The formation of these filaments does not necessarily result from a previous drying; it may occur also in the parasitised larva continuously submerged in water, but in this case the process takes place much more slowly. The successive drying and moistening of the spores appears, however, to hasten the formation of the filaments, and doubtless plays a very important part in nature, because the wound of the tree is necessarily exposed to alternating conditions of drought and moisture.

All the stages in the formation of these filaments are easily found by opening the body of the parasitised larva in a drop of water a few days after the insect's death (Text-fig. 2). The process whereby the barrel-shaped spore gives rise to filaments can only be followed, however, by examining smears prepared from the dead infected larvae, fixed with Schaudinn's solution and stained with iron-haematoxylin.

Such smears show very clearly that the filaments are the unrolled internal spirals liberated from the spores. The latter, under the pressure of the unrolling spirals, rupture at one end, and from the opening in the ruptured sporocyst a portion of the spiral protrudes (Pl. IV, figs. 42–47). The protrusion usually begins at one end of the spiral, which progressively unrolls and liberates itself from the sporocyst. At various stages of this unrolling the three central round cells are mechanically expelled from the spore, leaving the sporocyst completely empty.

In somewhat rare cases the protrusion of the spiral begins either with a loop (Pl. IV, fig. 44) formed by its central portion or with both extremities simultaneously (Fig. 46).

The same smears also contain many other spores devoid of a sporocyst (Text-fig. 3). The spiral filament of these spores is of a very irregular shape, the rings which form it being often loosened or the unrolled portion secondarily twisted. The irregular form of these filaments is undoubtedly due to mere mechanical pressure produced during the preparation of the smears.

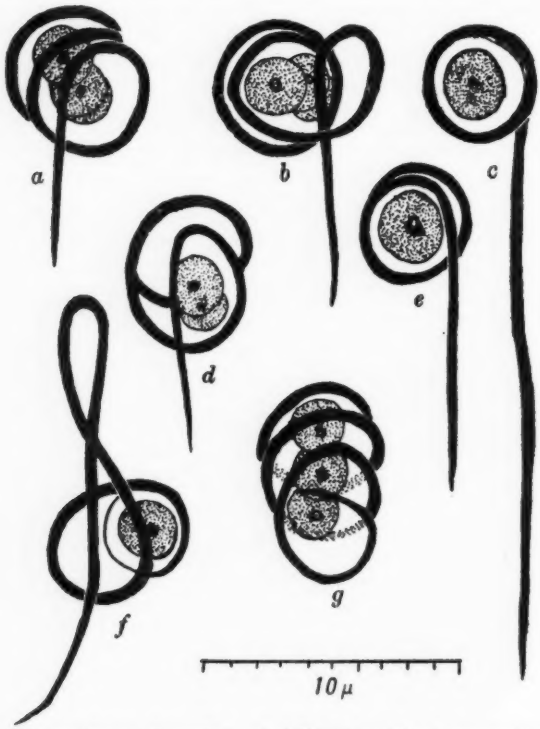
Text-fig. 2. A drop of fluid taken from the dead body of a *Dasyhelea* larva infected with *Helicosporidium*. The drop diluted with normal salt solution, shows different stages of the opening of the spores and unrolling of the spiral filaments.

The three liberated central cells, which are 2μ in diameter, remain much as they were when inside the sporocyst, with the difference that the refractive granules in their protoplasm are now more distinctly visible.

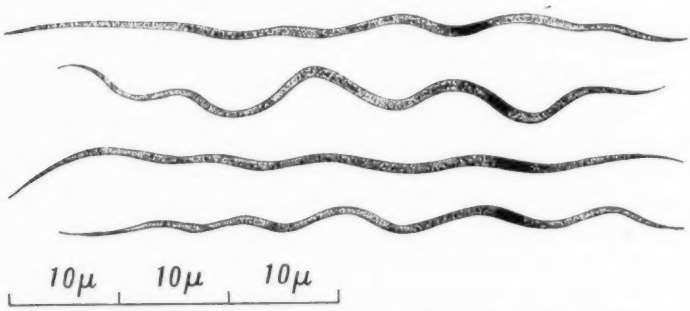
The structure and size of the filament are fairly uniform. Examined *in vivo* they are straight, needle-shaped (Text-fig. 2 and Pl. V, figs. 8 and 9), 60–65μ long and 1μ wide. They are very refractive, pointed at both ends, but the two extremities are not equally attenuated.

In fixed and stained smears, these filaments are more or less sinuous and much narrower, not exceeding $0{\cdot}65\mu$ in their widest portion (Text-fig. 4, Pl. V, fig. 7). This is undoubtedly due to the fact that the central axial

part of the filament takes the stain, while the peripheral refractive non-staining sheath either contracts or becomes invisible in refractive mounting



Text-fig. 3. Different forms of unrolling spiral filaments without sporocyst; such forms are often found in fixed and stained smears.



Text-fig. 4. Unrolled filaments in fixed and stained smears showing their nuclei.

media. In iron-haematoxylin the filaments stain very slowly, but once stained, they are very retentive of the dark colour and are decolorised with difficulty.

After differentiation in iron alum, the darkly stained axial portion of the filament presents a granular structure, and if the differentiation is more prolonged, until the filament becomes of a grey colour, a darkly stained chromatic body, of definite size and structure, makes its appearance (Text-fig. 4). This body, which is undoubtedly a nucleus, is 2–3 μ long and 0·6 μ wide, always lies in the wider portion of the filament, 15–18 μ from its extremity. By prolonged differentiation this nucleus can also be detected in filaments of the spiral form while still enclosed in the sporocyst (Pl. IV, fig. 41). We now arrive at the final stage of the life-cycle of the parasite: the distended cuticle of the dead larva being filled with a felt-like mass of entangled filaments mixed with small cells, the central cells of the spores and the empty sporocysts (Pl. V, figs. 8 and 9).

At this stage the macerated cuticle of the larva breaks away and its entire contents escape into the surrounding medium, the decomposed sap of the tree. Smear preparations of this sap often reveal the above-mentioned filaments, while the central cells of the spores cannot be recognised in the crowd of various micro-organisms, yeasts, moulds, rhizopods, ciliates, etc., which usually inhabit the fluid.

5. *Supposed mode of infection of the host.*

A question arises now: which is the infective form of *Helicosporidium*? Is it the filament or the central cells of the spore? The cellular structure of the filament, its great resistance to external influences, and its resemblance to the infective stage of *Monosporella* suggest that it may be the infective stage. If this is the case, the central cells of the spore could only be considered as residual bodies.

On the other hand, the great number of the central cells (three times more numerous than the filaments) and their resemblance to the first stages of *Helicosporidium*, as seen in the body-cavity of the host, make it almost certain that they represent the real sporozoites or infective forms of the parasite. In this case the spiral filament may be regarded as a cell differentiated for the purpose of dehiscence of the spore and can be compared in respect to its function with the elaters of Mycetozoa, with the difference that, while in the latter they are of complicated structure and extrasporal, in *Helicosporidium* they are unicellular and intrasporal.

The sporozoites, after being swallowed by a healthy larva, penetrate probably through the wall of the alimentary canal into the body cavity of the larva, where they begin their endogenic multiplication or schizogonic cycle.

6. *Frequency of infection of* Dasyhelea *larvae.*

Several generations of *Dasyhelea obscura* occur in the course of a year, and the larvae of all the generations are equally subject to infection. It is, however, impossible to estimate the true rate of infection as this varies greatly and depends upon the condition of the wound of the tree at the time

when the material is collected. In rainy weather the larvae leave the flooded parts of the tree's wounds and penetrate into the fissures of the tree; in the meantime, the wound is thoroughly washed by the rain and is freed from the collected sap which usually contains dead and dying larvae infected with *Helicosporidium*. When the normal conditions are restored and the wound is once more covered with freshly exuded sap, the larvae crawl again from their hiding places and invade the wound. If the sap is collected at this time, very few infected larvae will be found. On the contrary, in damp weather with the absence of much rain, when the old sap remains in the wound for a prolonged period, the number of diseased larvae increases. Finally, the sap collected from the wounds and kept in jars in the laboratory gives a still higher proportion of diseased larvae, as in this case the non-infected larvae rapidly become infected from contact with their diseased companions.

7. *Stages of the host susceptible to infection.*

In all its larval stages *Dasyhelea obscura* is susceptible to infection with *Helicosporidium*. This is undoubtedly due to the feeding habits of the larvae remaining uniform throughout its life. Very small larvae, hardly 1·5 mm. long, were often found with the body cavity filled with spores of the parasite, the spores showing already completely formed spirals and the three sporozoites. In a single instance a full-grown larva, almost ready to pupate, showed only the schizogonic cycle of the parasite, a condition which indicates a recent infection. Between these two extremes all the intermediate phases are met with. Only a few pupae of *Dasyhelea* were found infected and this infection is almost certainly derived from the larval stage: a full-grown larva became infected just before pupating, when all its imaginal discs were already completely formed and the pupation took place before the parasite had time to make a destructive invasion. Such infected pupae are eventually killed by the parasite. In no case have I observed the parasite in the adult insect.

III. Other Hosts of *Helicosporidium parasiticum*.

1. *Mycetobia pallipes* Meig. (Diptera, Rhyphidae).

The larvae of *Dasyhelea obscura* are usually found associated with a number of other Dipterous larvae living in the same medium. According to their feeding habits, these larvae can be separated into two groups:

(1) Saprophagous larvae which like *Dasyhelea* feed upon the decomposed sap *e.g. Rhyphus fenestralis* Scop., *Mycetobia pallipes* Meig., *Aulacogaster rufitarsis* Meig. and the larvae of *Eristalids*.

(2) Carnivorous larvae such as *Systenus adpropinquans* Loew, and *Phaonia cincta* Zett.

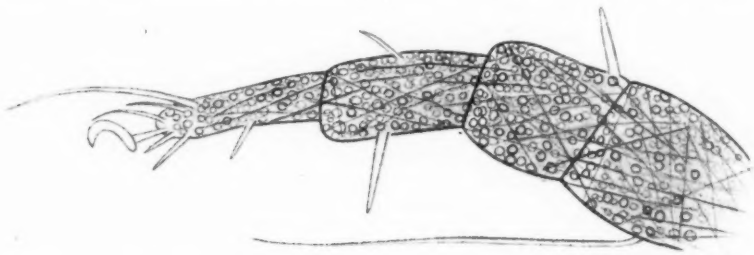
I have frequently examined large numbers of larvae of all these different species for various parasites and only once have I found the spores of

Helicosporidium in a specimen of *Mycetobia pallipes*, invading the peripheral portion of its fat-body.

2. *Hericia hericia* (Robin) Kramer (1899) (Acarina, Tyroglyphidae).

In addition to the above-mentioned Dipterous larvae, the exudate which fills the wounds of the elm tree contains also a very interesting mite belonging to the family Tyroglyphidae. This mite, *Hericia hericia* (Robin) Kramer, 1899, is undoubtedly the most frequent inhabitant of the exudate. It was discovered and very well described by Robin (1868) who found it in the exudate of elm trees in France.

The mite has been since found in England by Michael (1903, Vol. II. "Tyroglyphidae," pp. 31–38, Pls. XXIII–XXIV) who gives in his monograph of British Tyroglyphidae a complete description of all its stages. Concerning its habitat he writes: "This species usually lives in a semi-aquatic condition, wading in the sap which exudes from splits in the bark of elm trees, or under loose bark of these trees, and in the brown saccharine matter which collects there. In such situation it is often present in great numbers; it is also found,



Text fig. 5. A leg of *Hericia hericia* heavily infested with *Helicosporidium* showing the spores and filaments

but less frequently, in similar situations on the oak. I have not found it on other trees, but it is quite possible that it may exist on them."

I myself found this mite abundantly, often covering the entire surface of the wound.

On many occasions I have carefully examined large numbers of this mite without finding a single parasitised specimen, but recently (October, 1920), whilst collecting the *Dasyhelea* larvae, I noticed a portion of the body of *Hericia* with two legs attached to it, the whole filled with filaments and a few complete spores of *Helicosporidium*. I believed at first that I was dealing with an empty skin of a dead *Hericia* which was invaded by a small *Dasyhelea* larva parasitised and killed by *Helicosporidium*, but on careful examination of other mites, I soon discovered eight entire specimens, three of which were alive, all showing *Helicosporidium* in different phases of its life-cycle.

Text-fig. 5 shows a leg of a very heavily infested specimen of *Hericia*, the whole body of which is invaded with the spores and a large number of filaments.

All this shows clearly that *Helicosporidium parasiticum*, although a common parasite of *Dasyhelea obscura*, is by no means specific to this host, but occurs in other Dipterous larvae like *Mycetobia pallipes* and what is still more remarkable, in at least one other Arthropod, the Tyroglyphid mite *Hericia hericia* (Robin) Kramer, 1899.

IV. Systematic Position of the Genus *Helicosporidium*.

Now that we know the structure and the life-history of *Helicosporidium*, a question arises as to the systematic position of this genus. It seems to me very difficult to answer this question and all I can do at present is to discuss the relations between the new parasite and various forms of Protists.

1. Helicosporidium *and Cnidosporidia.*

One is tempted first of all to compare the genus *Helicosporidium* with the Sporozoa, especially those which have multicellular spores, as for instance Cnidosporidae, comprising the three Orders: Myxosporidia, Actinomyxidia and Microsporidia.

The trophozoite stage of *Helicosporidium* as well as its schizogonic cycle recalls in many respects those of the Microsporidia Monosporogenea Pérez (*e.g. Nosema bombycis*); on the other hand, the trophic stage, in the form of a small round cell, as well as the schizogonic cycle, cannot be used for establishing the affinities between the various groups of Protists as similar modes of multiplication can be found in widely separated orders.

On the other hand the development and the structure of the spores provide a series of much more important characters which have been used already with success in the classification of the Protozoa. A character which is common to all Cnidosporidia and *Helicosporidium* is the complicated multicellular structure of the spores, the latter in both cases being composed of heterogeneous elements. It remains to be seen, however, in how far the spore cells of *Helicosporidium* can be compared with those of the Cnidosporidia.

It may be assumed, for instance, that the three central cells or sporozoites of *Helicosporidium* correspond to the germ cells, sporoplasm or sporozoites of the Cnidosporidia, while the polar capsule of the latter corresponds to the spiral filament of our parasite.

It must be admitted, however, that this assumption, which at first sight appears to be reasonable, is based on very superficial points of resemblance, and it needs a critical examination. The germ cells or sporoplasm of the Cnidosporidia differ from those of *Helicosporidium* in that they are usually reduced to a single binucleated cell, instead of three uninucleated cells as in *Helicosporidium*.

Among the Cnidosporidia the uninucleated sporozoites are known in a few species of Actinomyxidia, but they are very numerous in each spore.

As to the filament of *Helicosporidium*, although it is formed from one

differentiated cell as is the polar capsule of Cnidosporidia, it differs from the latter in several essential characters.

The following table shows the points of difference between these two structures:

Spiral filament of *Helicosporidium*	Polar-capsule filament of Cnidosporidia
(1) Filament not enclosed in a polar capsule but lies free beneath the wall of spore.	(1) Filament enclosed in a capsule of which it forms a part.
(2) Filament always unrolls in the dead body of its host.	(2) Filament does not unroll until spores reach intestine of a second host.
(3) Filament unrolls slowly.	(3) Filament is shot out.
(4) Filament pointed at both ends and wide and ribbon-like in the middle.	(4) Filament pointed at one end only and very fine.
(5) Axial portion of filament is very chromatic, nucleus well formed in anterior third of filament.	(5) No chromatic axial portion, degenerated nucleus upon wall of terminal capsule.
(6) Filament robust, very resistant in all media.	(6) Filament fine and very fragile.

The foregoing shows clearly that the spiral filament of *Helicosporidium* is of a nature totally different from that of the polar capsule of the Cnidosporidia.

Other distinctive characters of *Helicosporidium* are: (1) the wall of the spore does not seem to be formed by a specialised cell, at any rate no trace of such a cell could be detected in the wall of the completely formed sporocyst; (2) the spore of *Helicosporidium* does not show a binary or ternary symmetry and pansporocysts are non-existent.

All this demonstrates clearly enough that there is no real affinity between the genus *Helicosporidium* and the Cnidosporidia.

2. Helicosporidium *and Haplosporidia.*

We may now compare the genus *Helicosporidium* with the Order Haplosporidia of Caullery and Mesnil (1905). Although this Order, conceived in its widest sense, is heterogeneous, all the forms which it comprises differ greatly from *Helicosporidium*. In their life-cycle they have a plasmodium stage and a cyst which surrounds the spores; these characters never appear in the life-history of *Helicosporidium*. The spores of the Haplosporidia are unicellular with one or two envelopes while the spores of *Helicosporidium* are composed of four cells of different structure. These differences are sufficient to show that there is no affinity between *Helicosporidium* and the Haplosporidia.

3. Helicosporidium *and* Serumsporidia.

It remains finally to be seen whether or not there are some relations between *Helicosporidium* and a few Protists temporarily placed in the Sporozoa but whose systematic position is still *subjudice*. The only group among these Sporozoa which may interest us is the group of Serumsporidia of Pfeiffer.

Under the generic name of *Serumsporidium*, Pfeiffer (1895) has described a certain number of parasites which he discovered infesting the body cavity

of several species of Crustacea, belonging to the genera *Cypris*, *Daphnia* and *Gammarus*.

The descriptions and figures of this author are, however, very superficial and incomplete, and it is hardly possible to get a general idea of the structure of the parasites which he has described.

Neither his descriptions nor his figures of some eight distinct species of this genus give the remotest indication as to the character which these species have in common. From his description one can only say that the few characters which are common to all his eight species are of no systematic value, namely (1) that these species live as parasites in the body cavity of Crustacea, (2) that they produce very numerous spores, (3) that their structure and life-cycle are equally obscure.

From the systematic point of view the term *Serumsporidium* has no more value than the term "blood parasites" applied to protozoal parasites of mammals.

It is almost certain that his "*Serumsporidium II* (Mülleri), nov. sp.," "*Serumsporidium gammari*" and especially "*Serumsporidium cypridis IV*, n. sp." are the spores of a gregarine liberated from the ripe cysts, which, being fragile, have become ruptured inside the body of the host.

From all his descriptions and figures those relating to his species "*Serumsporidium* (*Cytamoeba? Labbé*) *cypridis I*, nov. sp." (p. 12), are of especial interest to us, as three of the figures illustrating this species bear some resemblance to *Helicosporidium*. His figure 2, B. 7 (p. 12), for instance, recalls the morula of *Helicosporidium* composed of four cells, one of which is concealed behind the other three, while the Figs. 2, B. 8 and B. 9, resembles somewhat the spores of *Helicosporidium* which shows the three nuclei of the central cells only. It is, however, hardly possible to base upon these superficial resemblances any relationship between *Serumsporidium* and *Helicosporidium*.

4. *Helicosporidium and Mycetozoa.*

Helicosporidium has no affinities with the Mycetozoa, several species of which have been already found parasitic in insects. It differs from the Mycetozoa in the absence of the plasmodium and flagellate stages and by the complicated structures of the spores, which, on the contrary, are simple in Mycetozoa.

V. Conclusions.

The foregoing evidence shows clearly that the genus *Helicosporidium* differs markedly from all the actually known Protists, and that it forms a new type which may be temporarily included in the group of the Sporozoa. It is possible that the discovery of other new forms of Protists, parasitic in insects, will throw more light upon the systematic position of *Helicosporidium* or will lead to the finding of a connecting link between this new genus and the already well-known forms.

The genus *Helicosporidium* may be characterised as follows:

Helicosporidium n. g.

Diagnosis. Parasitic protist; trophic stage in the form of a small round cell 2–3 μ in diameter, with small spherical nucleus. Schizogonic multiplication very active. Schizonts forming a small morula of 4 μ in diameter, composed of four or eight merozoites, which become free.

Spore (5–6 μ in diameter) is composed of four cells surrounded by a thin wall or sporocyst. Of the four cells, three, which form the real sporozoites, are discoidal, occupying the centre of the spore. The fourth cell forms a peripheral spiral filament which surrounds the central cells.

The spores open inside the dead body of their host, by the unrolling of the spiral filament, and the sporozoites are thus liberated.

The spiral filament when unrolled is 60–65 μ long, pointed at both ends and 1 μ thick at its widest portion; its nucleus 2–3 μ long, lies 15 μ from one end of the filament. This filament is of a very resistant nature. One species known.

Helicosporidium parasiticum n. sp.

Diagnosis. The same as that of the genus.

Habitat. Body cavity, fat body and nervous system of the larva of *Dasyhelea obscura* Winnertz (Diptera, Nematocera, Ceratopogonidae). Occurs also in *Mycetobia pallipes* Meig. (Diptera, Nematocera, Rhyphidae) and in *Hericia hericia* (Robin), Kramer, 1899 (Acarina, Tyroglyphidae). The hosts inhabit wounds of elm and horse-chestnut trees, Cambridge, England.

Acknowledgements. I am much indebted to Mr L. E. Robinson, who is engaged in research in the Quick Laboratory, for the very friendly help that he has given me in the preparation of this paper for the press and especially for the great pains he took in the preparation of the accompanying photomicrographs (Pl. V).

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EXPLANATION OF PLATES IV—VI.

Helicosporidium parasiticum.

PLATE IV.

All the figures of this plate relate to the smears fixed in Schaudinn's solution with 1 per cent. of acetic acid added, and stained in iron-haematoxylin and eosin. Owing to the small size and complicated structure of the spores, high magnification was used for this study: Apochromatic imm. object. 2 mm.; N.A. 1·4 with Comp. oculars 8 and 12. For the sketches which were made with the camera lucida, comp. ocular 18 was used; the scale of magnification common to all the figures is shown in the plate beneath the figures.

Figs. 1, 2, 3. Trophic stages of parasite.

Figs. 4, 5. First division of the parasite and formation of two cells.

Figs. 6–9. Different forms of bicellular stage.

Figs. 10–12. 2nd division and formation of schizonts of four cells.

Figs. 13, 14. Schizonts composed of four cells, tetrahedrally disposed.

Fig. 15. Exceptional forms of four cellular schizonts with merozoites radially disposed.

Figs. 16, 17. Breaking up of four-celled schizonts into four merozoites.

Fig. 18. Nuclear division in schizonts of four cells.

Figs. 19, 20. Schizonts composed of eight cells.

Figs. 21, 22. Breaking up of eight-celled schizont into eight merozoites.

Figs. 23, 24, 25, 26. Three successive stages in formation of morula of four cells tending to formation of the spores.

Figs. 27, 28. Further transformation of these cells showing one cell growing more rapidly and surrounding the three other cells.

Fig. 29. Obscure stage showing a central mass surrounded by a ring.

Fig. 30. Hypothetical figure showing the three central discoidal cells surrounded by the fourth cell.

Fig. 31. A young spore, end view, showing a central darkly stained body composed of three superposed discoidal cells, surrounded by a very refractive ring, and by a thin wall or sporocyst.

Fig. 32. Side view of a similar spore showing clearly the three central cells.

Fig. 33. Further development of the spore (as shown in Fig. 32), showing the spiral filament formed in the refractive ring surrounding the three central cells or sporozoites.

Figs 34–37. Spores viewed side-wise, similar to that illustrated in Fig. 33, showing some variation in size (due to fixation) and in the appearance of nuclei of the central cells.

Figs. 38–40. End view of a similar spore, the figs. 39 and 40 do not show the sporocyst, this being due to contraction by the fixative.

Fig. 41. Spore stained and much decolorised, showing a nucleus in the spiral filament.

Fig. 42. Spore seen from one end in a smear where almost all the other spores are already open (derived from the dead larva).

Figs. 43, 44. Ruptured spores showing the protrusion of a portion of the filament.

Fig. 45. Protrusion of the filament through the opening of a ruptured sporocyst.

Fig. 46. A case of protrusion of both ends of the spiral filament.

Fig. 47. More advanced stage of protrusion of the filament and its unrollment.

PLATE V.

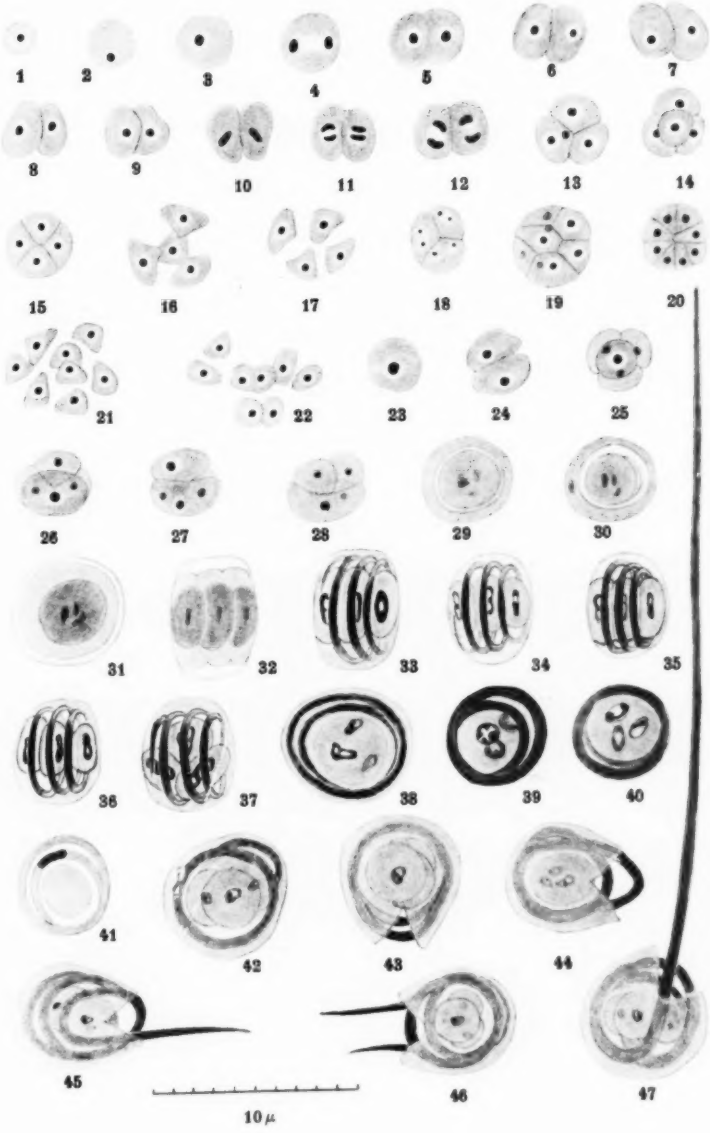
Photomicrographs taken by Mr L. E. Robinson.

Fig. 1. Living larva of *Dasyhelea obscura* heavily parasitised, pressed between the slide and a coverslip until the posterior end of its body was ruptured, projecting an enormous mass of spores. The latter can be seen still filling the body cavity of the larva and reaching into the head. × 75.

Fig. 2. The upper portion of the same larva under a higher magnification. × 150.

Fig. 3. Another larva, filled with spores of *Helicosporidium* and ruptured in its middle, showing the spores inside and outside the body. × 150.

Fig. 4 × 800. Fig. 5 × 300. Fig. 6 × 250. Fig. 7 × 500. Fig. 8 × 100. Fig. 9 × 400.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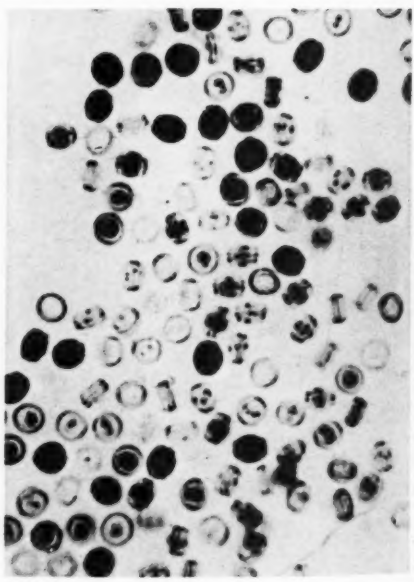

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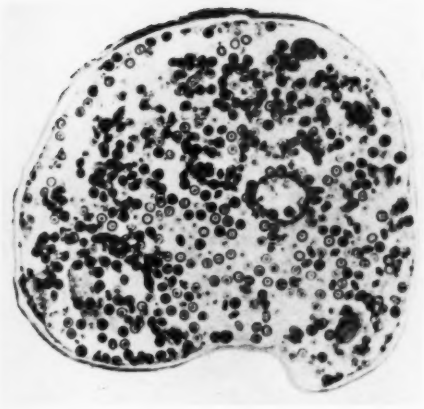
L. E. Robinson, photo.

PLATE V



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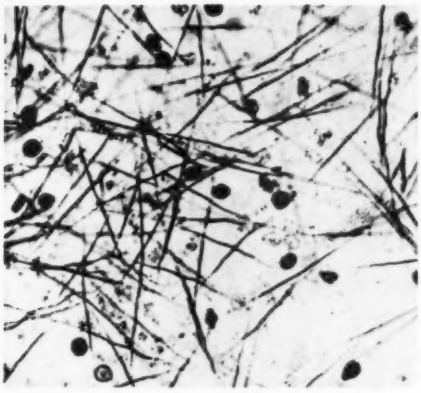

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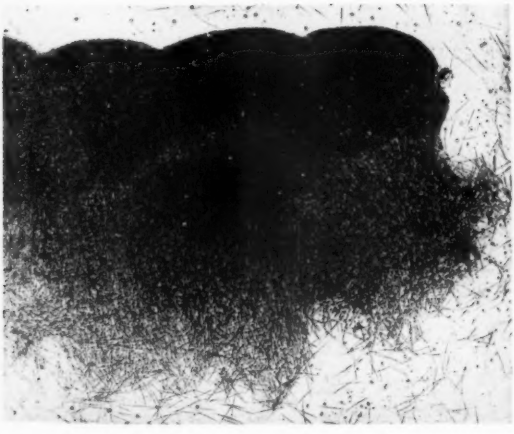

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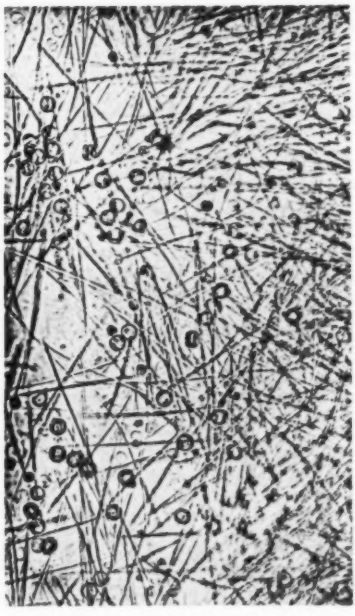

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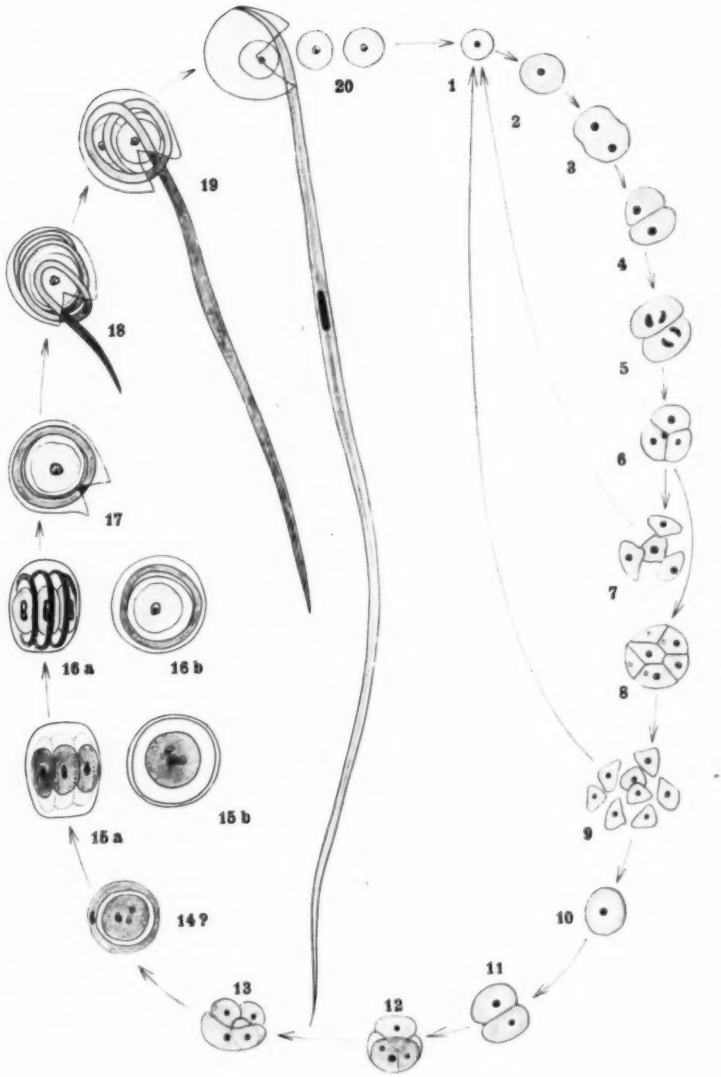

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Fig. 4. Smear, fixed and stained, showing the spores with central cells and spirals.
Fig. 5. Transverse section of *Dasyhelea* larva very heavily infected with *Helicosporidium*.
Fig. 6. Transverse section of *Dasyhelea* larva not heavily infected with *Helicosporidium*.
Fig. 7. Spores and unrolled filaments of *Helicosporidium* in fixed and stained smears.
Fig. 8. Dead body of *Dasyhelea* larva infected with *Helicosporidium*, the spores of which are open and the spiral filaments free and unrolled. The body of the larva is ruptured by the pressure of a coverslip and shows an enormous number of escaping filaments.
Fig. 9. A portion of the same mass of filaments under a higher magnification.

PLATE VI.

Life-cycle of *Helicosporidium parasiticum*.

Figs. 1–9. Different phases of schizogonic multiplication, formation of four- and eight-celled schizonts.
Figs. 10–13. Formation of four-celled morula which develops into the spores.
Fig. 14. Hypothetical stage showing the three central discoidal cells surrounded by the fourth cell.
Fig, 15. Young spore before the formation of spiral filament: (*a*) side view, (*b*) end view.
Fig. 16. Mature spore: (*a*) side view, (*b*) end view.
Figs. 17–20. Different stages of the opening of the sporocyst, unrolling of the spiral filament and liberation of sporozoites.

Stages 1–16 occur in the living larva of *Dasyhelea*, while the stages 17–20 are found only in the dead body of the host.

THE EXTERNAL ANATOMY OF THE *SARCOPTES* OF THE HORSE[1].

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Fellow of Trinity College, Cambridge.

(*From the Quick Laboratory, Cambridge.*)

(With Plate VII and 22 Text-figures.)

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INTRODUCTION.

Warburton (1920) has recently published a survey of our present knowledge of the mites of the genus *Sarcoptes*; from his survey it is clear that in spite of all the work that has been done upon these mites, much of it by most painstaking anatomists, we are still without an accurate knowledge of the anatomy of any one species of *Sarcoptes*. This is, in part at any rate, due to the fact that some of the best work was done, by Robin and others, between 1860 and 1865, at a time when microscopes and technique were by no means as highly developed as they now are.

This paper is an attempt to carry forward an investigation, the main lines of which have been indicated by Warburton; to describe as accurately as possible the anatomy of one *Sarcoptes*, that of the horse, and to illustrate its structure. A detailed knowledge of the structure of one species of *Sarcoptes* is desirable in order that the validity of the numerous very similar species may be settled once and for all. In this paper I purposely avoid reference to systematic

[1] Work done with the aid of a grant from the Ministry of Agriculture and Fisheries.

matters and deal only with the *Sarcoptes* of the horse, terming it *S. scabiei* var. *equi* Gerlach, without prejudice to the question of its rank as variety or species. I shall be glad to receive at the Quick Laboratory, Cambridge, specimens of *Sarcoptes* or other mange mites from any animal; the specimens should be sent alive if possible. When sufficient material has accumulated I hope to carry out a comparative study of the *Sarcoptes* of various animals.

I shall not deal with the life-history of *S. scabiei* var. *equi*. A good biological study of the closely allied, if separable, *S. scabiei* var. *hominis* has recently been published by Munro (1919), and, since it bears on my anatomical studies of the various stages of *S. scabiei* var. *equi* described in this paper. an outline of the life-cycle will be useful. From the egg hatches the larva, and from the larva emerges the nymph; I do not think that there is any external difference to be found between male and female. It is believed that the nymph, on moulting, produces either an adult male or an immature female. It is currently held that the immature female is the stage in which copulation occurs, but I have produced evidence (p. 139) for believing that in *Sarcoptes* the act is performed only by the adult female. We believe then that in the male there are three stages after the egg, and in the female four, that is to say the larva, the nymph, the immature female and the adult female.

THE ADULT FEMALE.

The following measurements are taken from ten females measured. Length from tip of chelicera to end of body, 357–432 microns (average 388 microns); breadth 250–295 microns (average 271). The ratio of length to breadth varies between 100/79 and 100/66, the average of the ten being 100/70. It will be seen that there are considerable individual differences not only in absolute size, but also in the relation between length and breadth. These differences are in all probability due to the action of the great longitudinal and dorsi-ventral muscles, which must be capable of producing great alterations in the shape of the mite.

Method. All the specimens were measured alive, and then cleared in gum arabic in order that I might satisfy myself by the examination of the tocostome that I was dealing with adult females. It is essential that this method should be followed in measuring *Sarcoptes* because it is readily compressed by a cover-slip, and altered in size and shape by fixation. According to the earlier authors there are considerable differences in size and shape between the various species of *Sarcoptes*, but we do not know how many individuals they measured nor under what conditions, so that it is doubtful whether the measurements of one author are comparable with those of another. The dimensions of *S. scabiei* var. *equi*, as given by Canestrini and Kramer, are length 450–500 microns, breadth 310–370 microns. These measurements are considerably in excess of my own, and were possibly taken from mounted specimens. Gerlach gives length 440, breadth 300 microns for the female, figures much closer to my own.

The body. The general shape of the body may be seen from Fig. 1. Subject to considerable individual differences it is roughly oval, and widest slightly behind the middle. It is irregularly convex above, flatter beneath and of a

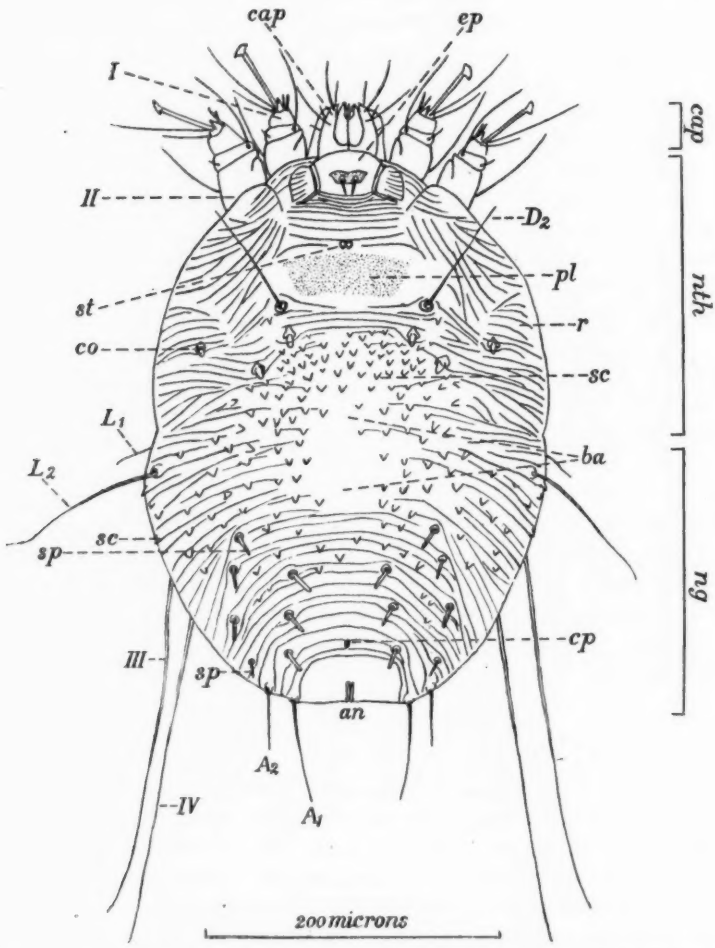


Fig. 1. *Sarcoptes scabiei* var. *equi*, dorsal surface of adult female. *A* 1, 2, anal setae; *an*, anus; *ba*, bare area; *cap*, capitulum; *co*, cone; *cp*, copulatory papilla; *D* 2, second dorsal seta; *ep*, epistome; *L* 1, 2, lateral setae; *ng*, notogaster; *nth*, notothorax; *pl*, plastron; *r*, integumentary ridges; *sc*, scale; *sp*, spine; *st*, ring-shaped rudiment of stigma; *I–IV*, legs. × 220.

translucent whitish colour, except where more strongly chitinized parts which lie on or near the surface are apparent by reason of their brown colour. The surface of the mite is covered with fine parallel ridges. One may generally

detect a division of the body into an anterior notothorax (*nth*), and a posterior notogaster (*ng*). The line which divides these parts of the body is slightly in front of the middle and runs across the ventral surface behind the tocostome;

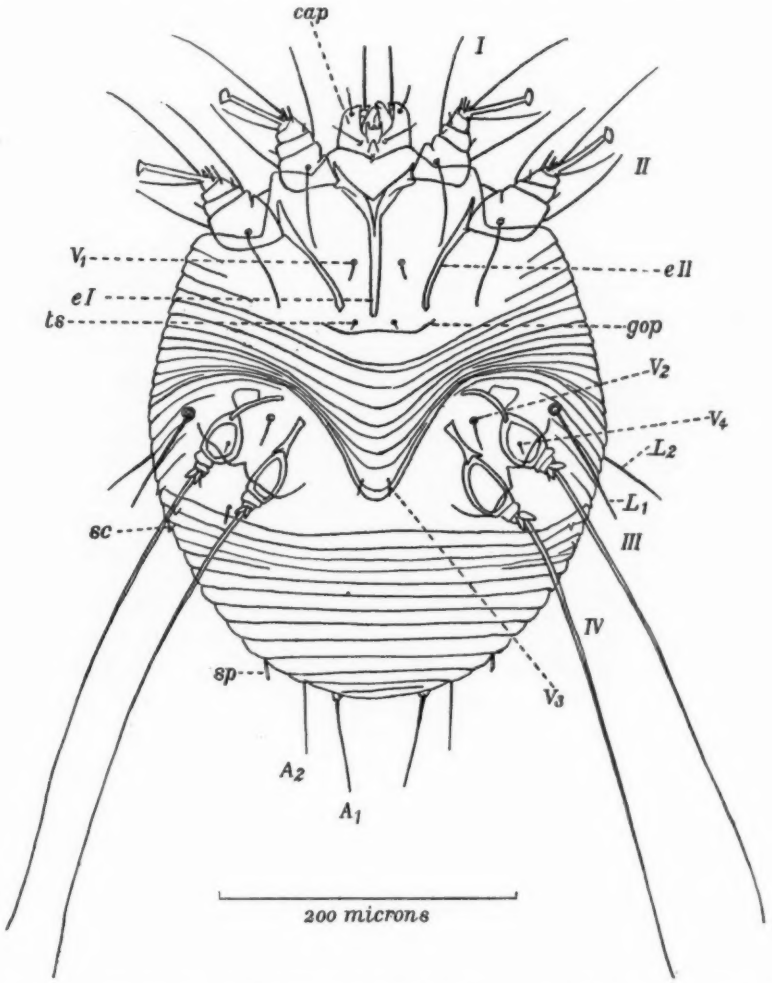


Fig. 2. *S. scabiei* var. *equi*, ventral surface of adult female. *A* 1, 2, anal setae; *cap*, capitulum; *e I–e II*, epimeres of legs; *gop*, genital operculum; *L* 1, 2, lateral setae; *sc*, scale; *sp*, spine; *ts*, tocostomal seta; *V* 1–4, ventral setae; *I–IV*, legs. × 220.

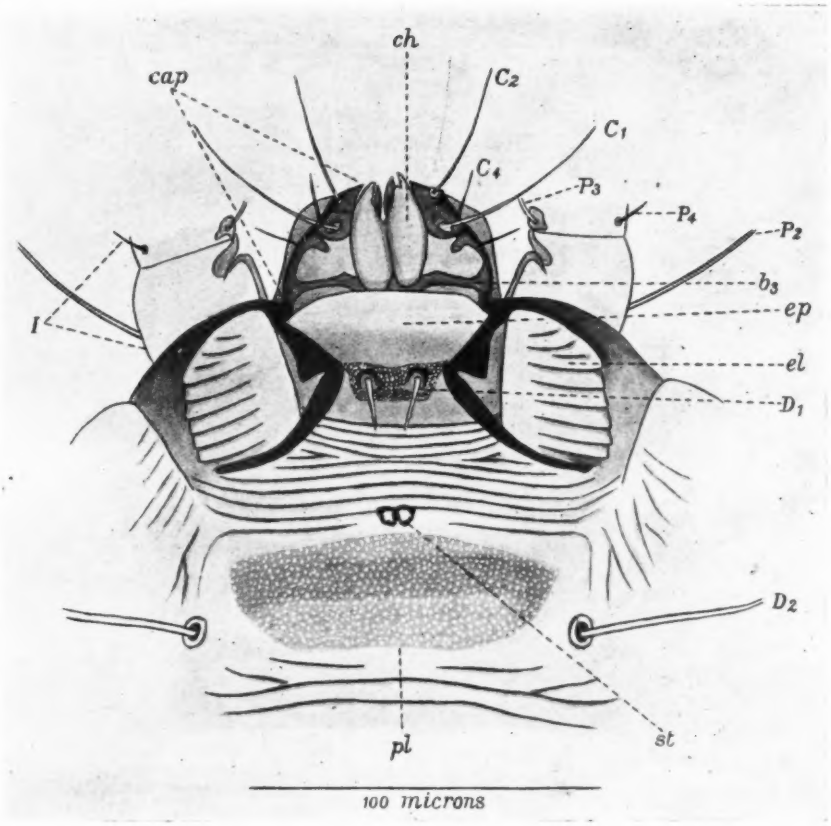
but its position is so indefinite that I shall endeavour to avoid the use of the terms notothorax and notogaster as far as possible. In living specimens in an advanced stage of pregnancy, when the egg has come forward to the tocostome,

the furrow between the notothorax and notogaster may be obliterated. It was from a specimen in this condition that Fig. 2 was drawn. The terms cephalothorax and abdomen which have been used for these two portions of the body are unsuitable because as Warburton says (*l.c.* p. 272) they bear a different meaning when applied to other groups of Arachnoidea; I might add that application of these terms to any Arachnoidea is open to objection.

The Dorsal Surface.

The dorsal surface (Fig. 1) is covered with fine parallel ridges which generally run transversely except where they follow the lateral contours of the body, or bend partially round the anus. A fold of the dorsal integument, the epistome (Plate VII, *ep*), covers the insertion of the basis capituli into the camerostome. The camerostome is a hollow in the front of the body for the reception of the capitulum. It cannot be seen from above. The epistome, which is in fact the dorsal wall of the camerostome, is not transversely ridged. Across its posterior part stretches a small area covered with shagreened sculpturing and containing two relatively large pits from which the setae (*D* 1) arise. This small shagreened area was noticed by Railliet in 1887 in *Sarcoptes laevis*, which is now placed in the genus *Cnemidocoptes* Fürstb., but it has not I think been described in any *Sarcoptes* in the restricted sense. Lobes of integument similar to the epistome cover the bases of the first and second legs; that covering the base of the first leg is particularly prominent, for the dorsal epimere surrounds a raised portion of the integument, the epaulette (*el*). There is no dorsal epimere and no epaulette in relation to the second leg. The ridges into which the greater part of the integument is thrown are faint and often interrupted in the mid-dorsal region over the greater part of the notogaster (see p. 119); they are absent from a small area on either side of the anus, and from a conspicuous roughly rectangular area on the notothorax. This area is the plastron of most authors including Railliet; the rugose area of Munro. (The "plastron" of Robin is on the ventral surface; it is the place of union of the first pair of epimeres with the sternum.)

The shape of this plastron (*pl*), as I shall call it, is shown on Plate VII. Its width is about $2\frac{1}{2}$ times its extent from before backward; the anterior margin is convex, the posterior straight; the anterior angles are acute, the posterior obtusely rounded. It is extremely difficult to represent this structure in a drawing, but I have endeavoured to do so in Plate VII. In Fig. 1 I have stippled it, so as to show its extent, but have not been able to indicate that the whole surface is shagreened; under an oil-immersion lens the appearance is as of a great number of points of light, regularly arranged upon a yellowish background. It is very slightly more strongly chitinized, and slightly browner in colour than the rest of the integument, but it is the easiest thing possible to overemphasize its degree of chitinization in drawing it. In fact the very word plastron is unsuitable, because it makes one think of an impenetrable plate of mail, but it is at any rate to be preferred to "rugose area," a term which is



Sarcoptes scabiei var. *equi*, anterior end of dorsal surface of adult female. *cap*, capitulum; *ch*, chelicera; *C* 1–4, setae ofcapitulum; *D* 1–2, dorsal setae; *el*, epaulette; *ep*, epistome; *P* 2–4, pedal setae; *pl*, plastron; *st*, rudiment of stigma (?); *I*, first leg. × 540.

The plate and all the text-figures have been drawn with a camera lucida.

definitely misleading, because the area is not rugose. Just anterior to the plastron there are two ring-shaped markings (*st*). These lie beneath the integument and are crossed by one of its folds. Their nature is entirely obscure. They consist of chitin and under high magnifications it is seen that their outline is slightly irregular. Mégnin described them as rudiments of stigmata; Munro figured them but did not mention them in his text; other authors, except Warburton, appear to have failed to notice them.

Behind the plastron half a dozen ridges pass across the dorsum and behind these there are a number of transverse rows of scales (*sc*, Figs. 1 and 3). These scales cover the greater part of the mid-dorsal surface; they are absent from the flanks and from the area in front of the anus. The rows on which they are arranged are continuous laterally with the ordinary ridges of the integument, and the scales represent a specialization of the ridge. Various stages in the perfection of the scale may be seen, from short blunt projections to the fully-developed organ, which is not thickly chitinized nor darker in colour than the integument from which it springs. In shape the scale is nearly equilateral, with a slightly concave base and very slightly convex sides;

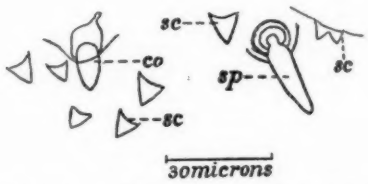


Fig. 3. *Sarcoptes scabiei* var. *equi*. On left, cones and scales from the notothorax; on right, scales and spines from the notogaster of an adult female; *co*, cone; *sc*, scale; *sp*, spine.

it is never acorn-shaped. It is difficult to count the scales owing to their irregular arrangement, but they number a little over one hundred. In Fig. 1 I have drawn an example in which the transverse rows are relatively regular, and in which traces of a longitudinal arrangement can be seen. The precise direction in which some of the smaller scales point is also variable, though the general direction of all is backwards. The largest scales are those on the lateral part of the notogaster.

In the example figured in Fig. 1 there is a single definite bare area (*ba*) in the middle of the dorsum. Its margins are ill-defined but from it scales and ridges are absent. In other adult females taken from the same horse this bare area is divided into two parts by a row or two of small scales passing transversely across it. The fact that this character is variable is of some importance because Railliet and also Canestrini and Kramer regard the presence of two bare areas as a specific character of *Sarcoptes scabiei* var. *equi*. As there is also variation in the number of scales present and in the regularity of the rows in which they are set I think we are justified in deciding that specific characters founded on them are of doubtful value. It is to these bare areas which occur

among the scales covering the notogaster that Railliet gave the name "clairière" in distinction to "plastron," by which he denotes what we call the plastron or rugose area. He remarks (1895, p. 641) with reference to *S. scabiei* Latr. that: "les écailles dorsales, chez la femelle ovigère, tendent en général à s'atrophier sur deux points, en laissant vers la ligne médiane une clairière plus ou moins nette; au niveau des épines postérieures du notothorax (clairière antérieure), et au niveau des épines antérieures du notogastre (clairière postérieure)."

It is probably to these areas that Canestrini and Kramer refer when they define *S. scabiei* var. *equi* as having "eine vordere und eine hintere Rückenblosse." Warburton is, I believe, mistaken when he states that "clairière," "blosse," and "plastron" have all been used for the same structure, the plastron on the notothorax.

Behind the plastron on each side are three cones (*co*), ("notothoracic cones," Munro, Warburton; "Schulterzapfen," Canestrini and Kramer; "épines courts," Railliet; "coni scapolari," Canestrini).

The antero-median cone lies behind the posterior angle of the plastron, the antero-lateral far out on the side of the body, and the posterior nearer the first than the second. The cone is considerably larger than the spine, acorn-shaped, covered at its base by one of the ordinary ridges of the integument, and articulated into a depression in the centre of an oval chitinous plate; this plate lies beneath the integument but is visible by reason of its brown colour.

On each side of the posterior part of the body there are seven blunt, lancet-shaped spines (*sp*). They are arranged in two longitudinal rows, a straight median one of three spines, and a curved lateral of four. It is convenient to designate this arrangement as 3 : 4. The posterior spine of the lateral row is finer and shorter than the rest. The spines are essentially similar to the cones and differ only in being articulated to a round plate of chitin, which appears to lie in, not beneath, the integument. The cones and spines differ from the scales in that instead of being merely specialized portions of the integumentary ridges, they interrupt the ridges and arise from independent chitinous plates; they are possibly specialized setae, and a consideration of the chaetotaxy of allied genera might show whether this is so.

The chaetotaxy is of systematic importance, and there is considerable disagreement between the figures of earlier writers; some of the smaller setae for long passed unnoticed, and not seldom ventral setae were observed by transparence and figured on both dorsal and ventral aspects. I have lettered every seta, and this will help with the comparative study of other species of the genus. Robin (1860) has already devoted attention to the comparative chaetotaxy.

On the dorsal surface there is a pair of short thick bristles (*D* 1) arising from pits in the small shagreened area which lies just in front of the epistome. Behind the posterior angle of the plastron a pair of very long setae (*D* 2) arise; these setae are directed outwards and forwards in the living mite when

it is removed from its burrow, but it is difficult to believe that they point in this direction when the mite is actually burrowing. On the side of the body there is a fairly long lateral seta (*L* 2) situated on a level with the posterior bare area. Warburton (*l.c.* p. 273) says that this is the "tasthaar" of Fürstenberg, but that author states (p. 181) that he uses this term to include all the larger setae of the body and legs. This seta differs from all others in that its origin is from a short but definite papilla. The base of the seta *L* 2 is latero-ventral, but the tip can be seen from above. On each side of the anus are two anal bristles; the median pair *A* 1 are longer and stouter than the lateral pair *A* 2; the median pair are strictly terminal, the lateral, distinctly on the dorsal surface, close to the edge of the body. It is difficult to say which are Munro's first pair of notothoracic bristles; if "they lie just below the camerostome" it is not easy to see why he attributes them to the dorsal surface at all.

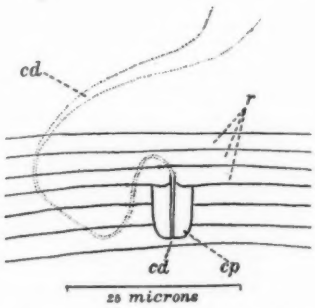


Fig. 4. *Sarcoptes scabiei* var. *equi*. Copulatory papilla of an adult female; the copulatory duct is visible through the integument and is shown with a dotted line; *cd*, copulatory duct; *cp*, copulatory papilla; *r*, ridges on the integument.

Immediately in front of the anus is an extremely minute papilla perforated by a fine tube which opens upon its summit. The papilla (*cp*, Fig. 4) itself is scale-like, flat and very freely moveable. The tube or copulatory duct (*cd*) passes from the summit of the papilla through its base, and penetrates deeply into the body. It appears to have a chitinous lining and can be traced for some distance, and it can be seen that it becomes gradually wider and wider. I shall return to a discussion of its function when I describe the male genitalia (p. 138), and the immature female (p. 139).

The papilla was first observed so long ago as 1861 by Gudden, who wrote an exceedingly interesting paper, dealing among other things with the internal and external genital organs of *Tyroglyphus* and *Sarcoptes*. He chose *Tyroglyphus* by reason of its larger size, and when he had familiarized himself with its internal anatomy he turned to *Sarcoptes*. He figures the whole genital tract of an adult female; it consists of the papilla, as I have described it, a narrow tube leading from it to a spherical spermatheca, and two divergent ovarian tubes which pass from the spermatheca to unite immediately behind

the tocostome or orifice by which they discharge the eggs to the exterior; at their point of union there is a lubricating gland. Gudden's results must have been due to pure dissection without the aid of the microtome, and are nothing less than astounding. I have made no attempt so far to investigate the internal anatomy of *Sarcoptes*, and I only find myself in disagreement with Gudden on one point; he figures the copulatory duct or vagina as a fine tube of even calibre passing from the copulatory papilla to the spermatheca; I find that it widens gradually and that this is the case in every specimen examined. Gudden's other results I accept with all humility. A year before the publication of Gudden's work the papilla had been seen and figured by Robin (1860); unfortunately he regarded it as a median spine, similar to the other notogastric spines except for its smaller size. On the whole Gudden's discovery has received very little attention though it is quoted in Railliet's text-book (1895). Mégnin (*a*) copies one of Robin's figures which show the papilla as a spine, though he makes no acknowledgement of the source of the figure and (1886) states that "les Glyciphages sont les premiers acariens chez lesquels nous constatons l'existence d'un organe speciale de copulation; chez les autres Sarcoptides, la copulation se fait par le fente anale, comme nous avons maintes fois constaté." Trouessart (1893) corrects this error, and says that his own observations support Gudden; he states that the male copulates with the immature female, and that it is in this stage that one best sees "la poche copulatrice"; the opening of the "poche," or as I should call it the papilla, looks like a hair-base from which the hair has been broken, but it is unpaired and median; the papilla is always referred to as being *behind* the anus, a misinterpretation which we must account for by supposing that in compressing specimens for microscopic examination the anus was brought round to the ventral surface. Munro (1919) and Warburton (1920) make no reference to the papilla nor to the discrepancy between Mégnin's views and the facts of the case; it appears that they overlooked the papers of Gudden (1861) and Robin (1860).

The anus (*an*) is a longitudinal slit at the extreme end of the body; it extends slightly more on the dorsal surface than on the ventral which is interesting because in the closely allied genera *Notoedres* and *Prosopodectes* it is distinctly dorsal in position. The anus is bounded on each side by a straight, thick lip. Fürstenberg figures the anus of various species of *Sarcoptes* not as a straight slit, but with a concavity towards one side or the other. This at any rate so far as *S. scabiei* var. *equi* is concerned is an error, produced probably by the study of slightly compressed specimens. Fürstenberg was on the whole a most careful man, but it is important to correct his errors because his figures have been reproduced widely. Robin (1860) and Railliet (1895) figure and describe it correctly.

The Ventral Surface.

The under surface of the body (Fig. 2) is nearly flat, and is crossed normally by the indefinite sulcus which divides the notothorax from the notogaster. It is covered by ridges of integument, transverse for the most part, but sweeping round the articulation of the posterior legs so as to become longitudinal; where the integument is strengthened by the ventral epimeres it is not thrown into ridges. At the anterior end of the ventral surface there is a deep V-shaped indentation. The sides of the V are the lower margin of the camerostome, and pass round the basis capituli somewhat like a collar. There are similar V-shaped indentations for the reception of the bases of the legs of the first two pairs. The ventral epimeres (*e I–IV*), are the most conspicuous objects on the ventral surface of the mite. In sections of the whole organism they appear as thickenings of its chitinous integument, extending into the body as ridges. They are to be regarded as specialized parts of the exoskeleton of the body, and not as parts of the leg; their function is to provide a firm *point d'appui* for the leg, and this they do by stiffening the exoskeleton which is elsewhere so soft. Their distal end is forked to receive the obtusely angular articular surface of the corresponding leg. One is tempted to think of them as ridges for the origin of muscles, as apodemes in fact; I am not prepared to say that they never fulfil this function, but it is certainly not their prime function. The epimere of the first leg (*e I*) passes backwards and inwards to meet its fellow of the opposite side at a point slightly behind the insertion of the basis capituli into the camerostome. From their point of union they pass backwards as a single chitinous structure, which terminates bluntly in front of the genital operculum (*gop*). This median structure, the product of the fusion of the first pair of epimeres, is the sternum of most authors, "sternal bar," and "sternal rod" of Warburton, "pièce commune ou sternale" of Robin. The name "plastron" is given by Robin (1860) to the point at which the first pair of epimeres unite.

The epimeres of the second pair of legs (*e II*), resemble those of the first pair in size, but differ in the greater development of the mesial limb of the fork which receives the articular surface of the basal joint of the leg; this limb of the fork is prolonged for a considerable distance up the space which separates the first and second legs (Fig. 8). The shaft of the epimere is curved towards the middle line, so that its basal part is nearly parallel to the sternum. The basal (posterior) end of the epimere is shown in Fig. 5. It bears a minute point and shows a very slight tendency to being forked. The epimeres of the third and fourth pairs of legs differ from the anterior ones in being much shorter. That of the third leg is curved so that its proximal end approaches the fourth. On its lateral side it is prolonged out into a thin semicircular flange, the free edge of which gradually passes into the chitin which covers the body. The fourth epimere is short, stout and nearly straight; it is shallowly forked at its proximal end, and more deeply distally, where it articulates with the fourth leg.

The following setae are present on the ventral surface: a pair of short setae (*V* 1) on either side of the sternum; a very short pair, the tocostomal setae (*ts*), close to the middle line on the base of the genital operculum; a pair of rather stout but short setae (*V* 2) arising from the bare space between the epimeres of the third and fourth legs; a pair of extremely fine setae inserted close to the middle line among the integumentary ridges which lie between the bases of the last pair of legs (*V* 3); and a short seta (*V* 4) on the cuff of integument which covers the base of the third leg.

In addition to these there are in the ventro-lateral region the setae *L* 1, which arise anterior to the dorso-lateral pair (*L* 2), and on a level with the epimere of the third leg. The seta *L* 1 is longer than any of the ventral setae but not so long as *L* 2. Other setae, which are visible on the ventral surface, but belong to the chaetotaxy of the legs are described on p. 130. The median

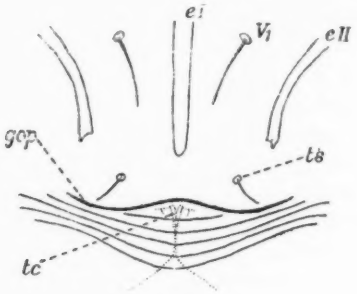


Fig. 5

Fig. 6

Fig. 5. *Sarcoptes scabiei* var. *equi*. Ventral view of the genital operculum and surrounding parts in an adult female. *e I*, *e II*, epimeres of first and second legs; *gop*, genital operculum; *tc*, tocostome; *ts*, tocostomal seta; *V* 1, first ventral seta.

Fig. 6. Ventral view of actual tocostomal orifice; the same structure as shown in dotted line in Fig. 5. *tc*, tocostome.

tocostomal seta which is figured by Fürstenberg and others, and apparently by Munro (1919), does not exist, at any rate in *S. scabiei* var. *equi*. As I shall explain below these authors have seen the longitudinal aperture of the tocostome, and figured it as a seta. All the setae on the ventral surface are directed backwards.

Three or four scales (*sc*) can generally be seen from below, on the flank at the level of the fourth pair of legs. The tip of the last spine (*sp*) of the outer row is also often in sight, projecting over the posterior end of the body, but it is of course a dorsal structure. With these exceptions there are no scales or spines on the ventral surface.

Behind the epimeres of the first and second pairs of legs a wide transverse flap, very shallow from before backwards, can easily be seen. This is the genital operculum (*gop*, Fig. 5), bearing at its base the tocostomal setae (*ts*).

This operculum[1] covers the actual orifice of the tocostome (*tc*) ("vulve," Robin; "vulve de ponte," Neumann), a longitudinal slit between two closely opposed slightly thickened lips. These lips lie slightly beneath the integument of the body, and are shown most clearly in Fig. 6. They are somewhat thickened in front where they are opposed to one another; further back they are more weakly chitinized and may be seen to diverge as they pass deeply into the body. In specimens which have been killed slightly before oviposition the lips may be seen widely separated to allow for the passage of the egg. There is therefore no doubt at all that this orifice is the tocostome, that is to say the birth opening or passage through which the egg is laid.

The earliest and at the same time the most accurate extant figures of the genital operculum are those of Robin (1860) and Gudden (1861). Since that time it has been figured and described by Delafond and Bourguignon (1862), and by Railliet (1895). Other authors seem to have failed to see it, and no one so far as I am aware has described the actual longitudinal orifice of the tocostome, except Gudden (1861). Railliet, for instance, speaks of "une fente transversale," obviously the line of opening behind the genital operculum. It is an extraordinary thing that a writer so recent as Munro (1919) failed to observe the genital operculum, and figures only a median structure which might be taken to represent either the opening of the tocostome, or the (non-existent) median seta first figured by Fürstenberg (1861).

THE CAPITULUM.

The structures which comprise the capitulum of *Sarcoptes* have been interpreted in a different way by almost everyone who has seriously tried to describe them. Modern technique and a modern microscope have helped me to produce the following description, which I believe is an improvement upon those previously published. I propose first to describe the structure of the capitulum and its component organs, and then to discuss certain points of interpretation and homology.

The capitulum (*cap*) consists of the chelicerae (*ch*) and palps (pedipalps) (*pp*), and the basis capituli or chitinous capsule to which they are attached. The general shape of the capitulum resembles that of a thumb-nail, as can be seen from Plate VII and Fig. 7; its length is about 60 μ, breadth 55, and depth from the dorsal to the ventral surface 50–60 at the base. Seen from the side the dorsal surface is flat, the ventral convex, and these approach one another from base to apex. The insertion of the basis capituli into the camerostome has already been described; the base of the capitulum is overlapped by the epistome above, and partly covered by the diverging limbs of the V-shaped opening below. The structure of the basis capituli, which is the name given to the whole capitulum except the appendages, is as follows. The chitinous

[1] The tocostome is generally overlapped by the margin of the genital operculum. In the example which I have chosen for figuring (Figs. 5 and 6) it lies slightly posterior to the margin of the operculum and is therefore more clearly seen.

investment of the basal part of the basis capituli is in some places thickened into bands which act as mechanical stays. The exact disposition of the bands on the ventral surface can be seen in Fig. 10. They support the base (*b* 1) and sides (*b* 2) of the organ; at its posterior extremity the bands *b* 1 unite at the middle line to form a rounded mass of chitin (*z*), the "menton" of Robin. The first epimere (*e I*) is in close relation to the side (*b* 2) of the basis capituli, but there is no articulation between them. Similar bands pass from the sides towards the middle line on the dorsal surface (*b* 3, Plate VII), and also on the

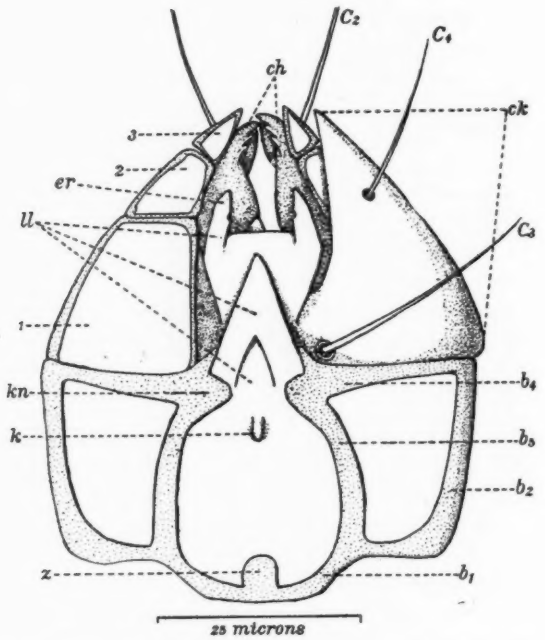


Fig. 7. *Sarcoptes scabiei* var. *equi*. Capitulum of adult female seen from below after removal from camerostome. *b* 1, 2, 3, 4, bands of chitin forming supports for the basis capituli; *C* 2–4, capitular setae; *ch*, chelicera; *ck*, cheek-piece; *k*, keel; *kn*, knob of chitin, the maxilla of Robin; *ll*, lower lip; *z*, part of the thicker chitinous skeleton of the basis capituli, the "menton" of Robin.

ventral (*b* 4). The inner end of *b* 4 meets the longitudinal *b* 5 to form a rounded heavily chitinized knob (*kn*). On the mid-ventral line there is a minute keel (*k*) projecting downwards.

The transverse bands *b* 3 on the dorsal, *b* 4 on the ventral side, divide the basal third of the basis capituli from the rest. From *b* 4 springs the cheek-piece (*ck*), a triangular plate of the thinnest and most colourless chitin, which covers the palp ventrally and laterally. This cheek-piece is so transparent that one has difficulty in seeing it when it is folded close about the palp. In

mounted specimens, especially those which have been exposed to some pressure, it is frequently pushed to the side; it then becomes more easily seen. The cheek-piece bears two setae on its ventral surface, *C* 3 a large stiff seta arising from a conspicuous pit near the mid-ventral line, and *C* 4, shorter and finer, arising close to the lateral margin of the cheek-piece near its extremity.

Between the cheek-pieces on the ventral surface is a complex structure which I prefer to call the lower lip (*ll*). It is the homologue of what is called the hypostome in ticks, but the term is slightly ambiguous. In the Ixoidea it is defined as a "median ventral structure arising from the basis capituli" (Nuttall, Warburton, Cooper and Robinson, 1911, p. 127), but in the rest of the Acarina it is used (*e.g.* by Hirst) for a ventral structure comparable to the epistome; in fact in this sense of the word the epistome and

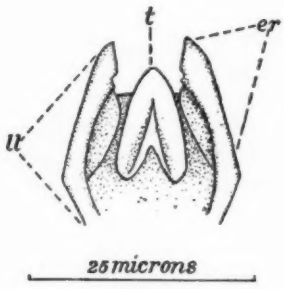


Fig. 8

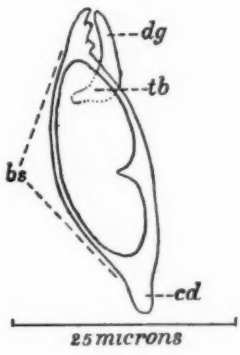


Fig. 9

Fig. 8. *Sarcoptes scabiei* var. *equi*. Dorsal aspect of lower lip of adult female. *er*, erect body; *ll*, lower lip; *t*, languette or tongue.

Fig. 9. Left chelicera, lateral aspect. The dorsal side is to the left. *bs*, base of chelicera; *cd*, condyle; *dg*, digit; *tb*, tubercle of digit.

hypostome are folds of the general body integument and lie respectively above and below the insertion of the basis capituli into the camerostome. The lower lip as I shall call this structure (Fig. 8), arises from the rounded knobs (*kn*) of chitin which as I have already said are formed by the union of the chitinous bands *b* 4 and *b* 5. It is a flat strip of chitin, for the most part thin and colourless, strengthened at its base by an acutely angled area of slightly thicker chitin. At each side of its extremity arises an erect body (*er*), which is immediately ventral to the chelicerae. These erect bodies are not articulated upon the extremity of the lower lip, but are simply continuations of its lateral margin; they are sharply excavated on their mesial aspects. Between these erect bodies the extremity of the lower lip is abruptly truncated. The tip of the structure known as the languette or tongue (*t*) lies upon the dorsal side

of the extremity of the lower lip, and may be seen projecting over its truncated margin. The appearance of the languette from above is seen in Fig. 8. It is a flat oval piece of chitin, deeply incised at the base, and thickened by a chitinous median ridge. It is analogous, if not homologous, to the hypopharynx of insects.

The palp (*pp*) (morphologically the pedipalp) is an organ lying on each side of the capitulum above the cheek-piece, below and to the side of the chelicera. In general shape it is cultriform, and when the two palps are flexed their terminal joints meet across the chelicerae. The palp is articulated to the base of the basis capituli over a wide area along the line of the chitinous band *b* 4; it consists of three joints, and the lines dividing them from one another are more clearly marked below than above. The first joint must be nearly immoveable, by reason of its articulation over a wide base to the basis capituli, and of its position between the chelicera above and the cheek-piece below; it is greater in length than the second and third joints combined, and is devoid of setae. The second joint bears the first capitular seta (*C* 1) on its dorsal surface; the third joint which is the shortest of the three and triangular in shape, bears the seta *C* 2 on its dorsal surface. The third joint terminates in a blunt point, without any of the delicate finger-like processes found in the closely allied *Psoroptes* (Buxton, 1920).

The chelicerae (Fig. 9) lie on the dorsal side of the capitulum, and are exposed for the greater part of their length. They lie above the palp and close to the middle line. Their general shape can be seen from Fig. 9. The chelicera is short; thick, slightly concave downwards and convex upwards. It consists of two portions, a base (*bs*) and a digit (*dg*). The base which forms the main part of the chelicera, bears a rounded articular surface or condyle (*cd*), which is in contact with a conspicuous articular surface inside the basis capituli. At its extremity it is decurved so as to form the upper limb of a chela, and it bears three teeth. To the mesio-inferior aspect of the base is articulated the digit (*dg*), which is not toothed but can be brought into opposition with the toothed extremity of the base; this completes the chela. All previous authors, except Robin, who give any attention to the matter figure and describe the digit as toothed. This is I believe due to an optical illusion; the whole chelicera is very small and when the chela is closed the teeth at the extremity of the base overlap the margin of the digit and impart to it a toothed appearance. The two parts of the chelicera are so jointed together that the tubercle of the digit lies free in the interior of the base. A muscle inserted on the tubercle and arising from the apodeme in the ventral side of the base would close the chela by bringing the two portions together. I have never been able to see such a muscle either in *Sarcoptes* or in *Psoroptes* in which genus I have recently figured a similar disposition of tubercle and apodeme.

So much for the description of the capitulum. There remain to be considered certain discrepancies between my interpretation and those of earlier writers.

Robin's drawings of the mouthparts, etc., of *Sarcoptes* are better than those of any of his predecessors or successors, but he made serious mistakes in interpretation. It appears that he was too much inclined to read a knowledge of insect anatomy into his observations on *Sarcoptes*. The result is that he regarded the pedipalps (or "palps" in the acarological but not entomological sense) as the homologues of the maxillary palp of insects; these pedipalps, or "palps" are segmental appendages in the strict sense of the word and therefore comparable to the whole of an insect appendage. Having made this mistake, a most natural mistake too in 1860, he searched for the rest of the "maxilla," and attached this name to the stout chitinous knob (*kn*) and the two chitinous bands (*b* 4, *b* 5) which unite to form it. In his definition of the Sarcoptidae he says (1860, p. 196): "á rostre pourvu de mâchoires inermes très petits portant des palpes maxillaires latéraux, voluminaux, à trois articles"; he adds that the "mâchoires ou maxilles" are "soudées ensemble par la ligne mediane."

Robin's mistake was copied by Railliet, whose figure was copied by Neumann, whose description repeats the error. The mistake does not merely affect our idea of the structure of *Sarcoptes* for this supposed union of the second appendage to form a median unpaired organ has become one of the accepted characters of the Sarcoptidae, and is figured as such by Berlese (1912, p. 11) in a figure which shows diagrammatically the supposed differences in the structure of the capitulum in different families of Acarina. I submit that this is an important, if academic, point, and that none of these authors produce evidence that the median unpaired structure is part of the pedipalp, or of any other appendage. It appears better to regard it as formed from the basis capituli, and to admit that we know nothing of its derivation in the embryo. It is not generally considered that any median ventral part of the head of spiders or scorpions, or other Arachnoidea represents a part of a pair of fused appendages, and it is not necessary to believe that this is the case in the Acarina.

I find myself in disagreement with Robin on a second point, which is of minor importance. The cheek-pieces are so thin and colourless that their mere detection is a matter of difficulty. Robin figures them fairly accurately but insists that they are the lateral lips of the camerostome, in fact prolongations of the body integument comparable to the epistome; I have convinced myself that they do not spring from the body at all, but are a part of the basis capituli itself, a ventral laminar prolongation of it arising from the transverse bar (*b* 4), and passing forwards beneath the palps. Fürstenberg (1861) figured and described the cheek-piece, which he called "backe," and his figure is at any rate accurate in this point that he derives the cheek-piece from the basis capituli . He made, as is well known, the extraordinary mistake of providing all his Sarcopts with two pairs of chelicerae; whether as Warburton suggests he once observed a *Sarcoptes* in process of moult and so became possessed by a fixed idea that there were four chelicerae, or whether he saw not only the extremity of the chelicerae but also of the erect bodies of the lower lip we do not know.

The Legs.

The anatomy of the anterior legs can be understood by reference to Figs. 10 and 11. The first and second legs are similar in all respects, as regards their size, chaetotaxy and their possession of five joints. More of the base of the leg can be seen from below than from above, owing to its ventro-lateral origin; the articulation of the first joint with the body is sunk in a pit, just as the

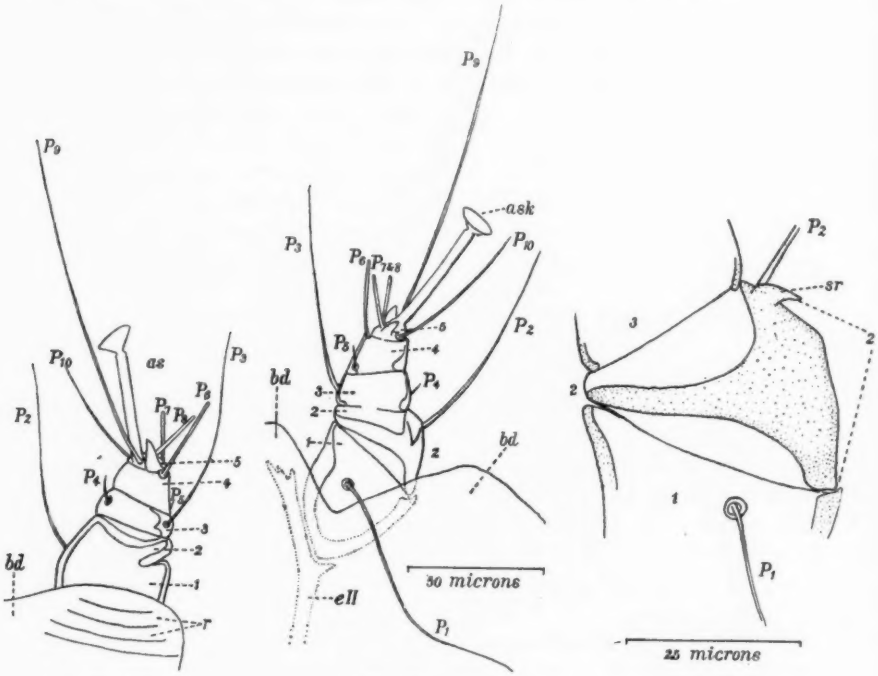


Fig. 10 Fig. 11 Fig. 11 *a*

Figs. 10, 11 and 11 *a*. *Sarcoptes scabiei* var. *equi*. Second left leg of adult female. Fig. 10, dorsal, and Fig. 11, ventral aspects. Fig. 11 *a*, ventral aspect more highly magnified, the leg slightly rotated so as to show the spur on the second joint. *as*, stem of ambulacrum; *ask*, its sucker; *bd*, body; *e II*, epimere; *P* 1–10, pedal setae; *r*, integumentary ridges; *sr*, spur on second joint ("trochantal spur"); 1–5, the five joints of the leg.

base of the capitulum is sunk in the camerostome. This point has apparently escaped notice hitherto, because it is difficult to detect the nearly transparent covering of integument with its wide V-shaped margin. In Fig. 11 *a* I have indicated with dotted lines the part of the first joint of the leg which is so covered. In Fig. 2 I have not dotted the corresponding lines, for with a low magnification one does not appreciate that these parts are covered at all. Actually of course they are extremely close to the surface. The first joint is

strengthened by a U-shaped, band-like thickening of its integument, the base of this U articulating with the fork at the distal extremity of the epimere, and the tip of each of its limbs with the second joint. On the ventral side of the joint a long seta (*P* 1) arises and passes backwards over the ventral surface of the body. The line of articulation between the first and second joints is oblique, and is apparent only on the under surface of the leg. On the upper surface the joints appear to be completely united, and it may be supposed that movement of the second article on the first is extremely limited; we must presume that there is some movement because as I have said there are definite articular surfaces between the ends of the U band of the first joint and the base of the second joint. On the mesial side of the leg there is an indentation, conspicuous from above and below, marking the line of the articulation between the first and second joints. On the under surface of the second joint (Fig. 11 *a*) is a T-shaped piece of chitin, the base of which articulates with the first joint; the head of the T lies upon the lateral margin of the leg; from it arise two structures, the seta *P* 2 which reaches as far as the tip of the ambulacrum, and the spur (*sr*), first described by Mégnin. This is a point of some importance for no writer since Mégnin's time, with the sole exception of Canestrini, has figured or described this structure, and some doubt has arisen as to whether it actually exists. Mégnin first observed it in *Sarcoptes* taken from the horse, and on this character and others he founded a new species *S. uncinatus*; when, however, he looked for the spur in *Sarcoptes* from other hosts he invariably succeeded in finding it. It is probably present throughout the genus, for Canestrini figures it (1894) in the species *dromedarii*, *leonis*, and *precox*.

The third joint is short, and on each side of it there is a longitudinal band-like thickening of the chitin. From each of these thickenings springs a seta; on the median side *P* 3 arises from a pit in a small chitinous plate, and on the lateral side *P* 4, a very short, stiff seta; both of these are on the upper side of the leg. The fourth joint is longer than the third, which it resembles in being stiffened by a mesial and a lateral region of relatively thick chitin. The mesial side of the joint is noticeably straighter than that of any other joint; from its base on the ventral side arises the minute seta *P* 5; the extremity of the joint is hollowed out; from the excavation arises *P* 6, a short stiff seta which does not taper to a point but is equally thick throughout its length. The fifth joint is short and may barely be seen among the multitude of structures which arise from it. The largest of these is the ambulacrum arising directly from the ventral side of the joint. This ambulacrum consists of a cylindrical chitinous stem (*as*), which suddenly narrows at its distal extremity where a cup-shaped sucker (*ask*) is attached to it by a narrow base. Fürstenberg figured a fine tube passing through the stem and opening in the concavity of the sucker. I believe that no such tube exists and that the error is due to an optical illusion such as is easily produced when one looks at a solid transparent cylinder, and I have confirmed my opinion by examining transverse sections

of the ambulacral stem, which proves to be a solid rod. The minute seta figured by Robin springing from the extremity of the ambulacral stem does not I believe exist. The ambulacrum as a whole is the "haftnapf" of Canestrini and Kramer.

The terminal joint of the leg is also armed with two claws. These arise from the proximal part of the joint, the one on the dorsal surface, the other on the ventral surface. Their shape can be seen in Figs. 7 and 8. Four setae also arise from this small and overcrowded joint. The first two (*P* 7 and *P* 8) are on the mesial aspect of the joint, close to the stiff seta (*P* 6), similar to it in shape, and slightly shorter. The third (*P* 9) arises close to the base of the ambulacral stem on the dorsal side of the leg; it is finely tapered and more than twice as long as the ambulacrum. The third (*P* 10) arises from the

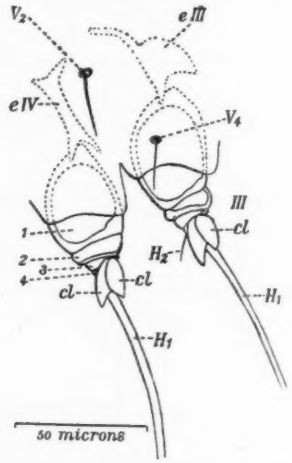


Fig. 12. *Sarcoptes scabiei* var. *equi*. Third and fourth legs of adult female seen ventrally. *cl*, claw; *e III–IV*, epimeres of third and fourth legs; *H* 1–2, setae of hind leg; *V* 2–4, ventral setae; 1–4, leg segments.

ventro-lateral aspect of the joint. It is about as long as the ambulacrum. The terms coxa, trochanter, femur, tibia and tarsus are used by many authors when referring to the five joints of the leg. This use is liable to the strong objection that the terms are technical names for certain parts of the insect leg, with which we cannot homologize the joints of the leg of the mites. As it is perfectly easy to speak of the "first joint" or the "fourth joint" I shall use this numerical terminology. Robin employs the terms hanche (rotule), exingual (trochanter), femoral, jambe, tarse, to designate the five joints.

The third and fourth pairs of legs (Fig. 12) resemble each other and differ from the first two pairs in the following respects; they are inserted as far from the middle line as the anterior are, but owing to the width of the body at this point they are completely concealed from above; they are smaller

than the anterior pair; they consist of four joints not five; they terminate in extremely long stiff bristles. If one examines either the fourth or the fifth leg one finds that the first joint is oval in shape, and covered for two-thirds of its length in a portion of the general integument of the body which is shaped like a cuff; the joint is about $2\frac{1}{2}$ times as long as the remaining three joints put together. On the cuff which covers the base of the third leg there is a short ventral seta (*V* 4). There is no corresponding seta on the cuff which covers the base of the fourth leg, though a seta in this position has been frequently figured. The second, third and fourth joints of either leg are successively smaller and smaller. The second and third joints are without setae; the fourth joint bears two claws, and the extremely long terminal seta (*H* 1) which corresponds to the ambulacrum of the anterior legs. This seta is equally long in the third and fourth pairs of legs. The terminal joint of the third pair of legs bears a minute seta (*H* 2) which is absent on the corresponding joint of the fourth pair of legs. (This seta is figured by Robin (1860) on both the third and fourth pairs of legs of *Sarcoptes scabiei* var. *hominis*.)

I can find no trace of a fifth joint though Robin (1860), who is an unusually careful investigator, describes the *five* joints of the posterior legs in detail. I believe he was misled by the fact that the second joint has a double outline, due to a transverse band of chitin which strengthens the extremity.

THE MALE.

The length of the male is 205 to 230 microns, the breadth about 170, and the ratio of length to breadth 100 to 75 or 85. The *dorsal surface* (Fig. 13) resembles that of the adult female except in the following particulars. The seta *D* 1 is shorter and stiffer than in the female; the plastron (*pl*) is relatively wider, and much longer, so that its length slightly exceeds its breadth, and the shagreening is coarser at the posterior end than at the anterior; only about 7 to 9 scales on each side are present. There is a pair of posterior plastrons (*ppl*) on the notogaster, between the dorsal and lateral rows of spines, and the shagreening of these is also relatively coarse. The shape of these can be seen from Fig. 13. There are only 12 spines (*sp*), arranged 3/3. The two pairs of anal setae (*A* 1 and 2) are present just as in the female: it must be due to an oversight that Munro (1919) figures one pair in his Fig. 3, two in his Fig. 6. The terminal seta *H* 1 of the third pair of legs is a conspicuous feature whether the mite is viewed from above or below, for it is as long as the body. The ambulacrum on the fourth leg is generally visible from above. There are three or four folds of integument on the side of the body on a level with the posterior plastron; they are very indefinite in number and degree of development.

The *ventral surface* (Fig. 14) differs very greatly from that of the female. The posterior end of the sternum or fused first epimeres extends considerably further back than a line joining the extremities of the second epimeres; these extremities are frequently described as forked, but it would be more true to

say that in shape they resemble a foot, with two sharp projections at the heel. The tocostome and its associated structures are of course absent. The ventral setae (*V* 1 to 4) and the lateral setae (*L* 1 and 2) have the same relative lengths as in the female, but *V* 3 is much more conspicuous, for instead of being concealed among integumentary ridges it is set on a flat place close to the epiandrium; the seta *L* 2 arises as in the female from a papilla, but its position is more distinctly ventral than in that sex. The third and fourth epimeres (*e III* and *e IV*) are united on each side to one another. The third is

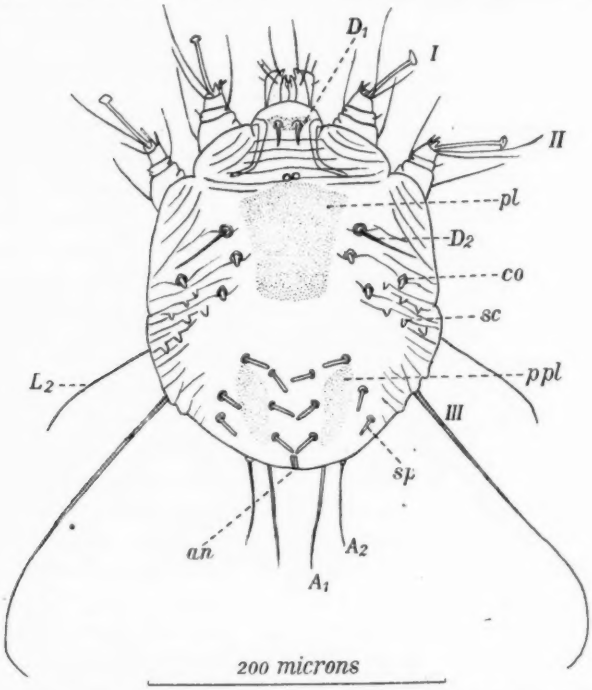


Fig. 13. *Sarcoptes scabiei* var. *equi*. Adult male, dorsal aspect. *A* 1–2, anal setae; *an*, anus; *co*, cone; *D* 1–2, dorsal setae; *L* 2, second lateral seta; *pl*, plastron; *ppl*, posterior plastron; *sc*, scale; *sp*, spine; *I*, *II* and *III*, legs (in the specimen figured the fourth leg is not visible from above).

very much bent, nearly transverse in position and provided with a wide flange; the fourth is straight and short. At the point at which they are united with one another, they have a definite surface by which they articulate with the epiandrium. In order to show this clearly I have very slightly separated these structures in Fig. 14. The epiandrium and the male organ is discussed separately below at some length. The *capitulum* of the male is similar to that of the female except that on the under surface the seta *C* 3 is slightly and *C* 4 considerably longer. In Fig. 13 the cheek-pieces have been displaced laterally

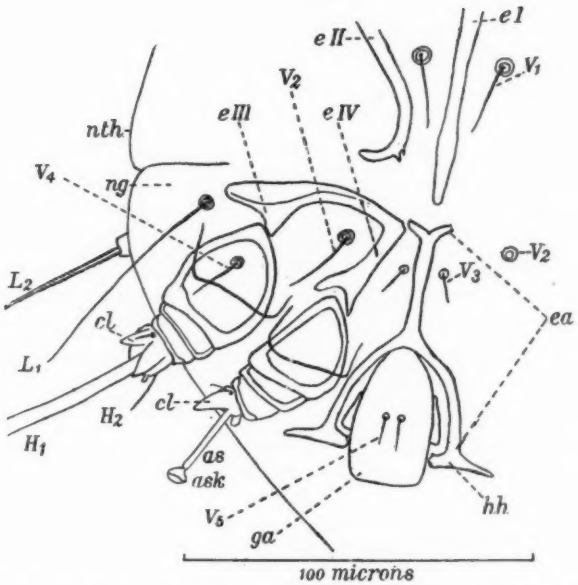


Fig. 14. *Sarcoptes scabiei* var. *equi*. Terminal part of ventral surface of adult male. *as*, *ask*, ambulacral stem and sucker; *cl*, claw; *e I–IV*, epimeres; *ea*, epiandrium; *ga*, genital apron; *H* 1–2, setae of hind legs; *hh*, hammer head; *L* 1–2, lateral setae; *ng*, notogaster; *nth*, notothorax; *V* 1–5, ventral setae.

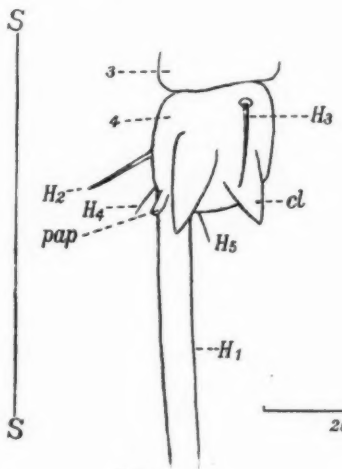


Fig. 15

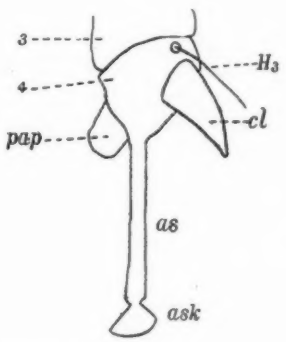


Fig. 16

Figs. 15 and 16. *Sarcoptes scabiei* var. *equi*. Terminal joints of third and fourth legs of adult male, seen ventrally. *cl*, claw; *H* 1–5, setae; *pap*, papilla; *SS*, sagittal plane; 3 and 4, third and fourth joints of leg.

and are therefore more noticeable than is usually the case; this is not a character of males in general. The first and second pair of *legs* resemble those of the female but the chitinous thickenings on the various joints are more strongly developed, so that the division of the leg into joints is more apparent. The chaetotaxy of the male and female anterior leg is identical except for the greater development of the minute seta *P* 4 in the male. As regards the spur, the claws and the ambulacrum, the male cannot be distinguished from the female. The third and fourth legs resemble those of the female in general structure as can be seen from Fig. 14, but differ in a number of small points. The seta *H* 1 of the third leg is extremely long, equal to the total length of head and body; *H* 2 is longer and stouter than in the female; in addition there are three setae, *H* 3, 4, and 5, which do not occur on the female; of these *H* 3 is a very small and fine seta, lying lateroventrally at the base of the lateral claw; *H* 4 and *H* 5 are short fine setae lying distal to *H* 2 and *H* 3 respectively. There is also present a minute papilla (*pap*) on the mesial side of the joint, distal to *H* 2. The fourth leg can at once be distinguished from that of all other stages by its possession of an ambulacrum in the place of the terminal seta; this ambulacrum is about half the size of the ambulacrum on the anterior legs. The setae *H* 2 and *H* 3 are present in the same relative positions as on the third leg; *H* 4 and *H* 5 are not present. The mesial claw is absent. In its place there is a large soft papilla.

Between the fourth epimeres of the two sides lies the complex genital apparatus. The most anterior part of this is the median rod of the epiandrium (*mea*), the "sternite" of Robin (1860). The anterior end of this rod is forked, and the limbs of the fork articulate on each side with a flat surface on the united third and fourth epimeres. In Fig. 14 the epimere and epiandrial rod have been separated by pressure in order to display the articular surfaces. The posterior end of the epiandrium consists of two curved epiandrial limbs (*eal*), springing from the median epiandrial rod. These limbs are the episternite of Robin; they diverge and pass backwards to terminate as two transversely placed hammer-heads (*hh*). The whole of this structure, the median rod, the limbs and the hammer head form the epiandrium (*ea*, Fig. 14), an organ which is comparable to an epimere; that is to say it is a rod-like internal thickening of the chitinous investment of the body, and its function is to supply a firm base for the erection of the genital organs themselves. From the concavity between the epiandrial limbs arises a flat lamella, the genital apron (*ga*), in shape something like a finger nail. This apron fills nearly the whole space between the epiandrial limbs, and exceeds that space posteriorly. It is an extremely thin, colourless sheet of chitin, bearing on its ventral surface a pair of small setae (*V* 5). Though it is quite transparent I have omitted from Figs. 14 and 17 the structures which lie beneath it, in order to simplify the figures. In nature the structures shown in Fig. 17 would be superimposed on these in Fig. 18. The parts which lie beneath the genital apron are shown in Fig. 18. In the concavity of the epiandrial limbs lies a horse-shoe shaped structure,

the hyposternite of Robin. It consists of three portions, the outer and inner rings, and the lateral rod. Of these the outer ring (*or*) is the largest, and at its posterior end is articulated with the hammer head. Part of the concavity

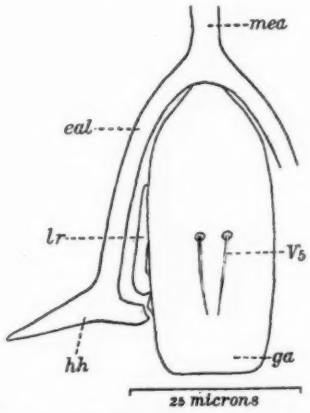


Fig. 17. *Sarcoptes scabiei* var. *equi*. Male genitalia seen from below. The structures behind the genital apron are not drawn, though actually the apron is quite transparent. *eal*, limbs of the epiandrium; *ga*, genital apron; *hh*, hammer head; *lr*, lateral rod of hyposternite; *mea*, medial epiandrial rod; *V* 5, fifth ventral seta, without counterpart in the female.

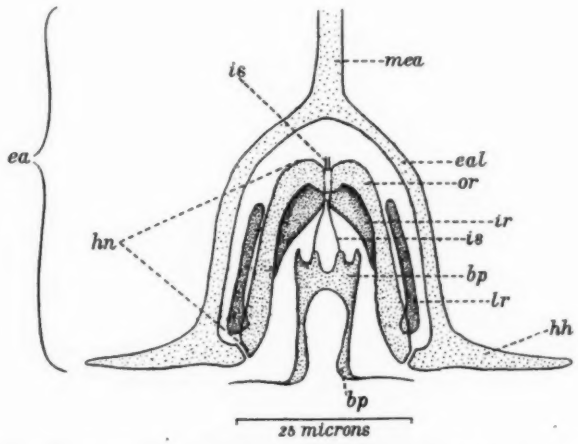


Fig. 18. *Sarcoptes scabiei* var. *equi*. Epiandrium as seen after removal of genital apron. *bp*, basis penis; *ea*, epiandrium; *eal*, its limbs; *hh*, hammer head; *hn*, hyposternite; *ir*, inner ring; *is*, intromittent spicules; *lr*, lateral rod; *mea*, median part of epiandrium; *or*, outer ring.

of the outer ring is filled by the inner ring (*ir*), which is intimately connected with the outer and lies on its dorsal surface. Through the most anterior part of both outer and inner ring passes a tubular passage (shown in dotted line

in Fig. 18); this passage opens in front at the bottom of a cup-shaped depression on the outer ring, and behind in the concavity of the inner ring. This passage contains the intromittent spicules. The third piece of the hyposternite, the lateral rod (*lr*), lies at the side of, and dorsal to, the outer ring. As I have said the intromittent spicules (*is*) pass through the passage in the inner and outer ring of the hyposternite, and their tips can be seen in the cup-shaped hollow at its end. They arise from a chitinous body, the "penis" of Robin; as I do not believe that this is an intromittent organ, I shall call it the basis penis (*bp*). It is shaped like a castle, with four projections like battlements upon its free anterior margin. It is from the two median projections that the intromittent spicules arise. At its posterior end the basis penis is wider and passes gradually into transverse folds of the integument.

The manner in which these very complicated genital structures are employed is not certainly known; so far as I am aware no one has ever observed *Sarcoptes* in copulation, and we must presume that the act is performed very rapidly; the closely allied *Psoroptes* is often seen walking about paired and the sexes do not separate when they are killed in alcohol. The current belief is that the female orifice for the reception of the male organ is within the anal opening; this is a fallacy (see p. 121). I agree with Gudden (1861) and Trouessert (1893) that copulation is performed through the minute opening on the copulatory papilla, and I believe that the duct which can be traced from this opening for a long way into the interior of the body leads to a spermatheca: I hope at a later date to deal with the internal anatomy of *Sarcoptes* and so settle this question; at present it is a matter of conjecture. The manner in which I believe the male organs are used is as follows. The whole of the epiandrium is a thickened part of the body wall; it therefore supplies a fixed base for the movement of the other parts. The hyposternite, hinged as it is to the hammer heads on each side can only move in one direction and is erected[1] so that it stands vertically to the surface of the body (or to the plane of the paper, in Fig. 16). The erection of the hyposternite would cause the erection of the basis penis, for they are locked together by the intromittent spicules; there is no difficulty in supposing this, for the basis penis is so attached to the body of the mite that clearly it can move in the sagittal plane of the mite, and in no other plane. When the hyposternite has been erected so as to stand perpendicularly to the ventral surface of the mite it appears probable that the copulatory papilla of the female is engaged in the cup-like hollow on the summit of the outer ring of the hyposternite. This brings the orifice on the papilla directly opposite to the tips of the intromittent spicules, which are I believe by this device enabled to find the extremely minute orifice. One has further to suppose, for I admit that it is a matter of supposition, that the vas deferens opens at some point on the summit of the basis penis, and that the spermatozoa

[1] I have failed to produce such erection by manipulating dead males, either freshly killed or macerated in water. Anyone who has attempted to manipulate the genital of a creature 225 microns long will understand why I failed.

pass along the intromittent spicules to the copulatory orifice and duct by capillarity. The weakness of this explanation is that it is from beginning to end a matter of hypothesis; its strength is that it explains the many peculiarities of the parts concerned, such peculiarities for instance as the minuteness of the spicules of the male, and of the papilla and duct of the female, and the presence of the cup-shaped hollow on the outer ring of the hyposternite.

THE IMMATURE FEMALE.

The length of the immature female is 230–250 microns, the breadth about 195, and the ratio of length to breadth about 100 to 80. It is therefore slightly larger than the male and a trifle wider on the average than male or female at any stage; as I have already stated there is a good deal of variation in these ratios, and I do not think that they have any practical value as a means of separating the stages. The line of division between the notothorax and notogaster is very indistinct or absent, in this and in the other immature stages. The *dorsal surface* (Fig. 19) resembles that of the adult female in nearly every respect. The dorsal epimere of the first leg is very weakly chitinized, and can only be found with difficulty; it is probably owing to its imperfect condition that an epaulette is not developed. The plastron (*pl*) is represented by a bare rectangular area, smooth and unfurrowed but differing from that of the adult in being not darker in colour than the surrounding integument and not shagreened or sculptured. The rudimentary stigma of Mégnin (*st*) is present. The dorsal armature is conspicuous because the individual cones (*co*), scales (*sc*) and spines (*sp*) are as large as those of the adult female. The scales stand in rather definite rows which are interrupted on the dorsum by the bare area (*ba*): this differs from the bare area of the adult in that though it is bare of scales it is crossed by integumentary ridges. The spines (*sp*) are in all respects similar to those of the adult, and the formula is 3/4; the last spine of the outer row is thinner than the others; possibly Munro overlooked it for he says that there are twelve spines in the immature female of *S. scabiei*. The dorsal lateral seta (*L* 2), and the two anal setae (*A* 1 and 2) are similar to those of the female.

The copulatory papilla and its duct are absent, an undoubted fact for I have examined a considerable number of immature females. This is very subversive of the accepted view that in the Sarcoptidae the immature female is the stage in which copulation takes place; the most likely explanation is that in the genus *Sarcoptes* it is the adult female alone which copulates. It is an exceedingly unfortunate fact that no one has as far as I know observed the act in this genus, and for the moment we must leave the matter in doubt. It is to be hoped that further light may soon be shed on this problem, which is one of the many interesting ones that remain to be solved.

The *ventral surface* is like that of the adult except in the following points: the genital operculum, tocostome and its setae are absent; the posterior end of the sternum is definitely forked, as is also the end of the epimere of the second leg. The *capitulum* and *legs* are in all respects like those of the adult

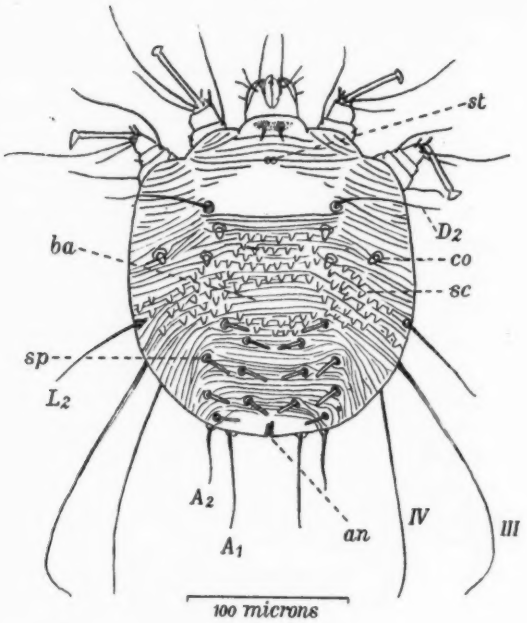


Fig. 19

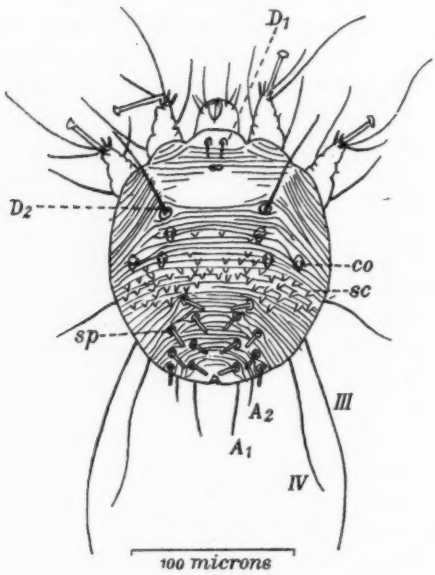


Fig. 20

Figs. 19 and 20. *Sarcoptes scabiei* var. *equi*. Dorsal views of immature female and of nymph respectively. *A* 1–2, anal setae; *an*, anus; *ba*, bare area; *co*, cone; *D* 1–2, dorsal setae; *L* 2, second lateral seta; *sc*, scale; *sp*, spine; *st*, probably rudiment of spiracle; *III* and *IV*, terminal setae (*H* 1) of third and fourth legs.

female; owing to their lower degree of chitinization it is not easy to distinguish the joints, and the skeletal rings and bands of thicker chitin; all these structures are, however, present as in the adult. The length of the long seta (*H* 1) of the third leg is about 80–82 per cent. of the body length.

THE NYMPH.

I am unable to find that the few nymphs I have seen fall into two groups, and can find no support for the statement that there are small nymphs which produce males and large ones which produce females. It is possibly the case but I have no evidence that it is so.

The nymph is about 175 microns long, by 135 broad, which gives a ratio of length to breadth, of 100 to 77.

The *dorsal surface* (Fig. 20) differs from that of the immature female in the following respects. The epistome and the seta *D* 1 are normal, but the small sculptured area from which *D* 1 springs in the adult and immature female is very indistinct in the nymph. The cones scales and spines are all smaller than in the adult and immature female. The scales, though smaller, are well formed and pointed, unlike those of the larva, and are just as numerous as in the immature female, but they do not extend across the dorsum posterior to the bare area, the posterior edge of which is therefore indefinite. The plastron is like that of the immature female, and differs from that of the adult female. The species are arranged as in the adult, the formula being 3/4. *A* 1 and 2 are present just as they are in the adult female. (Munro states that there is only one anal seta in the nymph of *Sarcoptes scabiei* var *hominis*.) Only one lateral seta (*L*) is present. From its position it is probably *L* 1, but I am not certain of this.

The *ventral surface* resembles that of the immature female and larva in the forking of the posterior end of the sternum and second epimere, a point which is shown in Fig. 21 *B* of the ventral surface of the larva, and in the absence of the tocostome and its associated structures; it differs from that of the immature female in the absence of setae *V* 3 and 4.

The *capitulum* is identical with that of the immature and adult female in all respects. The *legs* differ from those of the immature female in the absence of the long seta *P* 1, and in the fact that *P* 7 and 8, the short stiff setae on the fifth joint, are represented by a single seta. In respect of the spur, the claws, and the details of the joints the legs are identical with those of the adult. The posterior legs are also quite normal. The terminal seta (*H* 1) of the third leg is equal in length to about 82 per cent. of the total length.

THE LARVA.

The length of the larva is 140–160 microns, the breadth about 100 microns and the ratio of length to breadth as 100 to 66.

The characters of the *dorsal surface* (Fig. 21 *A*) are as follows. The seta *D* 1 which springs from the base of the epistome is identical with that of the adult,

but the small sculptured area is absent. The plastron is as in the other immature stages, that is to say it is represented by a smooth rectangular space the surface of which is free from shagreening. Only two cones are present, the absent one being the anterolateral. The scales are few in number, small and blunt. The bare area is like that of the nymph, crossed by ridges, and only enclosed by scales in front and at the sides. Only five pairs of spines are present, and the formula is 2/3. As in the nymph one lateral seta only (*L*) is present.

The *ventral surface* (Fig. 21 *B*) shows the following features: the presence of *V* 1 and *V* 2 and absence of *V* 3 and *V* 4, as in the nymph; the forked end of the sternum and second epimere as in the nymph and immature female; the presence of a single anal seta (*A*) on each side and the absence of the fourth leg.

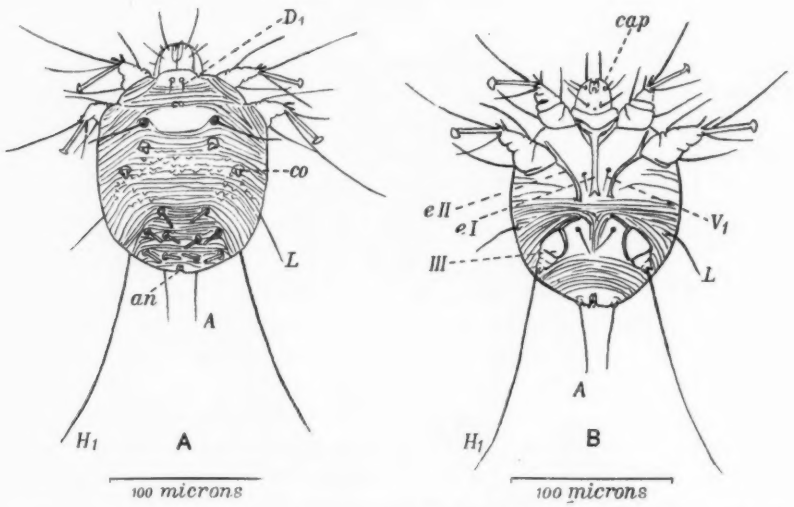


Fig. 21. *Sarcoptes scabiei* var *equi*. *A*, dorsal, and *B*, ventral views of larva. *A*, anal seta; *an*, anus; *cap*, capitulum; *co*, cone; *D* 1, first dorsal seta; *e I–II*, epimeres; *H* 1, terminal seta of third leg; *L*, lateral seta; *V* 1, first ventral seta; *III*, third leg.

The *capitulum* is in all respects like that of the other stages. The *legs* are like those of the nymph, in the absence of *P* 1, and the presence of a single seta in place of *P* 7 and 8. In other respects they are normal. The fourth leg is absent. The ratio of the length of the seta *H*, of the third leg to the total body length is 89 per cent. to 97 per cent.

The gradual appearance of the adult characters through the larval, nymphal and immature female stages is perhaps worthy of being summarized. The capitulum and its setae appear to be identical in all stages, though I must confess that I have made no dissections of the capitulum of the early stages. The principal setae, *D* 1 and 2, *V* 1 and 2, *A* 1, and the general shape of the

leg, and of its several joints is the same in the larva as it is in the adult female. In the following respects the larva differs from the nymph; it lacks the fourth pair of legs, and the second anal bristle; its dorsal armature consists of only two cones and five spines, on each side, and of a relatively small number of scales. The nymph and larva lack the setae *V* 3 and 4, and *P* 1 and either *P* 7 or *P* 8, and one of the lateral setae (*L* 2?), but in other points resemble the immature female. The larva, nymph and immature female resemble each other but differ from the adult principally in the absence of the tocostome, and its setae and of the genital operculum; but also in the forking of the sternum and second epimere, the ridging of the bare area, the imperfect

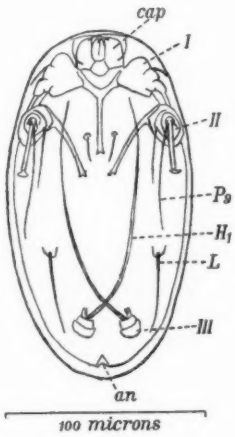


Fig. 22. *Sarcoptes scabiei* var. *equi*. Egg, in which is seen the ventral surface of a larva which is ready to emerge. Only some of the most conspicuous setae are shown. *an*, anus; *cap*, capitulum; *H* 1, terminal seta of posterior leg; *L*, lateral seta; *P* 9, ninth seta of second leg; *I*, *II*, *III*, legs.

separation of notothorax and notogaster, and the absence of shagreening on the plastron. It is therefore true to say that the assumption of the adult characters occurs gradually at each moult, and that no one moult more than another produces very marked changes.

THE EGG.

The length of the egg, measured without compression is 167–175μ, the breadth 88–97μ, and the ratio of length to breadth 100 to 51·5–56·8. The surface of the egg is smooth, without sculpturing. The example figured (Fig. 22) is about to hatch and the enclosed larva is seen by transparency.

KEY TO THE STAGES OF *Sarcoptes scabiei* var. *equi*.

The following key will probably be found useful; the egg stage is not mentioned.

A { Legs six. 150μ long ... ... Larva
Legs eight ... ... B

B { Fourth legs with ambulacra. Posterior epimeres united. Three plastrons. Length 225μ ... ... Male
Fourth leg terminating in long seta C

C { Tocostome and its setae present. Plastron shagreened. Length 390μ Adult female
Tocostome absent. Plastron not shagreened ... ... D

D { Bare area indefinite posteriorly. One lateral seta. Length 175μ Nymph
Bare area closed behind. Two lateral setae. Length 240μ Immature female

TECHNIQUE.

I have not found it necessary to develop a complicated technique. The following notes may be of service.

Collection. Scrapings should be made deeply to include the scurf and scabs next the skin. If the scabs are thick and only the superficial layer is collected it will be found to contain many larvae and very few of other stages. The scrapings may be folded up, as a doctor folds a medicinal powder, in a piece of paper. The mites migrate on to the paper in a very few hours; black paper therefore should be used so as to make them conspicuous. If the material cannot be examined fresh it may of course be preserved, or fixed, *en masse*.

Examination. It is essential to choose a fluid with the lowest possible refractive index, in order to render the sculpturing of the integument, and the fine setae transparent. Water is on the whole the most useful medium in which to examine specimens. Permanent mounts should be made in gum arabic (below). These mounts are as permanent as those made in balsam. The mite is placed in the gum alive, and dies rapidly in an extended position. Spirit specimens may be mounted in gum arabic, provided they are carefully washed in water. Levulose and lactophenol are also useful media, with lower refractive indices than gum arabic. They are unsatisfactory because they never harden, but at any rate they make useful semi-permanent specimens. Balsam is useless because it "clears" the specimen to such an extent that the chaetotaxy can only be made out with the utmost difficulty if at all.

The formulae of the mounting media are as follows. Gum arabic medium: distilled water 50 c.c., glycerin 20 c.c., gum arabic 40 gm., chloral hydrate 50 gm., cocain hydrochlor, 0·5 gm.; dissolve gum in water, add chloral hydrate and cocain, when dissolved add glycerin; filter if necessary. Levulose: saturated aqueous solution, evaporated to syrup on water bath, a trace of thymol added as preservative. Lactophenol: phenol crystals (puriss.) 1 gm., lactic acid 1 gm., glycerin 2 gm., water 1 c.c.

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ABBREVIATIONS.

The following abbreviations are used throughout the paper, in the plates and the figures:

A, anal seta of larva; *A* 1, *A* 2, anal setae of other stages; *an*, anus; *ap*, apodeme; *as*, ambulacral stem; *ask*, ambulacral sucker; *b* 1–*b* 5, chitinous bands of basis capituli; *ba*, bare area; *bd*, body; *bp*, basis penis; *bs*, base of chelicera; *C* 1–*C* 4, capitular setae; *cap*, capitulum; *cd*, condyle of chelicera; *ch*, chelicera; *ck*, cheek-piece; *cl*, claw; *co*, cone; *cp*, copulatory papilla; *ct*, copulatory tube; *D* 1–*D* 2, dorsal setae; *dg*, digit of chelicera; *e I–e IV*, ventral epimeres; *ea*, epiandrium; *eal*, epiandrial limbs; *el*, epaulette; *ep*, epistome; *er*, erect body on margin of lower lip; *ga*, genital apron; *gop*, genital operculum; *H* 1–*H* 5, setae of hind legs; *hh*, hammer head; *hn*, hyposternite; *ir*, inner ring of hyposternite; *is*, intromittent spicules; *k*, keel; *kn*, knob; *L*, lateral seta of larva; *L* 1–*L* 2, lateral setae of adult; *ll*, lower lip; *lr*, lateral rod of hyposternite; *mea*, median epiandrial rod; *ng*, notogaster; *nth*, notothorax; *or*, outer ring of hyposternite; *P* 1–*P* 10, pedal setae, on first two pairs of legs; *pap*, papilla on legs III and IV of male; *pl*, plastron; *pp*, palps; *ppl*, posterior plastron; *r*, integumentary ridges; *sc*, scales; *sp*, spine; *sr*, trochantal spur; *SS*, sagittal plane; *st*, ring-shaped organs, possibly rudiments of stigmata; *t*, tongue, or languette; *tb*, tubercle of digit of chelicera; *tc*, tocostome; *ts*, tocostomal seta; *V* 1–*V* 5, ventral setae; *z*, the "menton" of Robin; *I*, *II*, *III*, *IV*, the first, second, third and fourth legs; 1–5, the joints of palp or leg numbered from base.

ON THE *SARCOPTES* OF MAN[1].

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(With 9 Text-figures.)

Our knowledge of the systematics of the genus *Sarcoptes* is in an unsatisfactory state. About a score of species have been admitted, many of which have never been seen since they were described. Many of the descriptions date from between sixty and seventy years ago, a period when microscopes and microscopical technique were barely adequate to cope with so difficult a genus as *Sarcoptes* is. In order to provide a basis for further study of *Sarcoptes*, Warburton (1920) published a critical survey of our knowledge regarding these mites. His review of the literature has proved invaluable to myself, and is the foundation on which I have worked. In the foregoing paper (pp. 114–145) I published an only too lengthy description of the *Sarcoptes* of the horse, finding that a full description of one form was necessary as a basis for comparison. Since we do not know what anatomical points are of systematic importance, attention was given to all details of the external anatomy.

I propose to deal now with the *Sarcoptes* of man. It is commonly held that the "itch" is caused by a species or variety peculiar to the human race (*Sarcoptes scabiei* var. *hominis* (Hering)) and that this mite is separable on anatomical grounds from the sarcopts of other animals; it is believed that man is also attacked by "*S. scabiei-crustosae*" Fürst., and that this mite, which produces symptoms graver than those of itch, is a "good species," separable from *S. scabiei* and from the species attacking quadrupeds by definite anatomical characters. This second sarcopt is however very little known, and the "Norwegian Itch" or "Crusted Scabies," of which it is the cause, is a rare disease.

1. *Sarcoptes scabiei* var. *hominis* (Hering).

In comparing the common *Sarcoptes scabiei* of man with that of the horse particular attention has been devoted to the characters on which previous authors separated their species and the following conclusions have been reached. Canestrini and Kramer give 300–450 by 250–350 microns as the dimensions

[1] Investigation carried out with the aid of a grant from the Ministry of Agriculture and Fisheries.

of female *S. scabiei*, and 450–500 by 310–370 microns as those of the sarcopt of the horse. Gerlach separated his "*S. equi*" from *S. scabiei* on its greater size and greater relative length in the ovigerous female. I have shown (*loc. cit.*) that variation in size and proportions occurs even when mites are measured living under standard conditions. The relation between width and breadth is altered by pregnancy. My material of the common *S. scabiei* was collected by Mr J. E. M. Mellor during the war from a large number of different cases both civil and military, therefore the possibility is excluded that we are dealing with a case of equine scabies in man. I have been unable to obtain sufficient living material from man to give the measurements of a series of specimens, but I do not believe that differences of size are of the least use in separating species and varieties of *Sarcoptes*. The plastron in adult females from man is frequently, but by no means always, less highly pigmented than it is in those from the horse. There is no difference to be detected in the three plastra of the male. The bare areas (rugose areas) vary in their development among

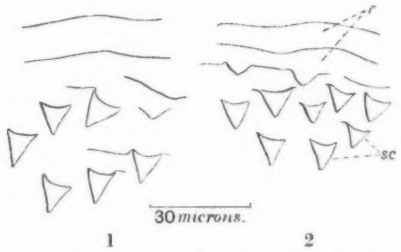
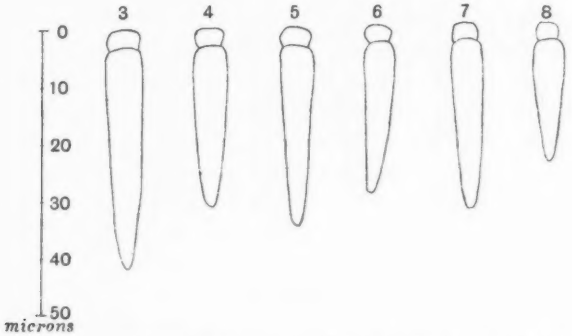


Fig. 1. *Sarcoptes scabiei* var. *hominis*, adult ♀. Scales from left side immediately behind the antero-median cone.

Fig. 2. *S. scabiei* var. *equi*, adult ♀. Scales from same area. The two figures drawn to the same scale with a Zeiss camera lucida. *r*, integumentary ridges; *sc*, scales.

specimens from man just as they do among specimens from the horse. In ovigerous females from either host either none or one or two may be present, or a large one partly divided by a row or two of scales; if two are present one or other may be very small. Neither the plastron nor the bare areas are of any systematic importance. The scales in specimens from man are said to be pointed and longer than broad; in those from the horse, small and scarcely longer than broad (Canestrini and Kramer). Hirst (1920), speaking of specimens from man, refers to the "dorsal scales being distinctly longer as compared with their breadth than in *Sarcoptes scabiei* var. *equi*." I find that the differences are so slight, and subject to such individual variation, that they are useless for purposes of discrimination. Scales vary greatly in size on any one mite, but the largest on ovigerous females from the horse do not exceed 8·5 microns in length. In specimens from man they reach 10–11 microns, as a maximum, but on many mites no scale longer than 9·5 can be found. Figs. 1 and 2 show scales from the same part of the dorsum of females from the horse

and from man. In shape I can detect no differences provided that a sufficient number of scales be examined. Scales are slightly concave or slightly convex at the side, or concave on one side, convex on the other; these variations occur quite irrespectively of the host from which the specimen was derived. Canestrini and Kramer state that the spines of *S. scabiei* var. *hominis* are long and pointed, those of var. *equi* blunt. This distinction is borne out by measurements, and I have measured the second spine of the inner row, and the second spine of the outer row of a series of ovigerous females. That of the inner row measures 31–33 microns in *Sarcoptes* from the horse, that of the outer row 25–28 microns: corresponding figures for *S. scabiei* var. *hominis* are 35 microns and 29–30 microns. There is a further small and rather inconstant difference in the greater stoutness of many of the spines of the equine



Figs. 3 and 4. *Sarcoptes scabiei* var. *scabiei-crustosae* (Fürst.), adult ♀. Second spine of inner (3) and outer (4) rows.

Figs. 5 and 6. *Sarcoptes scabiei* var. *hominis*, adult ♀. Second spine of inner (5) and outer (6) rows.

Figs. 7 and 8. *Sarcoptes scabiei* var. *equi*, adult ♀. Second spine of inner (7) and outer (8) rows. All the figures are drawn to the same scale with a Zeiss camera lucida.

Sarcoptes. The slight curve in Fig. 6 is due to the spine being viewed laterally; all spines are slightly curved, and the curvature cannot be used for separating races. I can detect no other differences whatever between *S. scabiei* var. *hominis* and var. *equi*. I have examined with particular care the cones, the trochanteric claw, and the chaetotaxy of the capitulum, body and legs; taxonomic differences have been described in all these structures, but I can find no differences whatever. I have examined adult males and females, but not the immature stages.

I conclude that the *Sarcoptes* of the horse and the common species found on man cannot invariably be separated. Certain minute differences exist in scales and spines but they are not constant, and the measurements overlap. Other characters which have been used for separating these forms appear to be wholly useless. It is convenient to regard these forms as varieties, as many

previous writers have done; this is more justifiable on physiological than on morphological grounds. The common itch mite of man stands as *Sarcoptes scabiei* de Geer, 1778, var. *hominis* (Hering, 1880); that of the horse as *S. scabiei* var. *equi* (Gerlach, 1857).

2. "*Sarcoptes scabiei crustosae*" Fürstenberg.

Fürstenberg differentiated a species under this name, giving as its character its shape, which is "rounded, slightly longer than broad," and the greater length of one of the lateral setae, almost certainly the seta which I have lettered *L* 2. Canestrini and Kramer say "dorsal scales blunt; no bare area (Blösse). Spines long, acute, slightly bent. Anterior arm of epiandrium strongly developed and reaching the epimere. Male 170 × 150 microns, female 410 × 340."

I have examined a series of specimens obtained by Dr Wallace Beatty from a case of Norwegian crusted scabies, and kindly lent to me by Col. A. Alcock, F.R.S., I.M.S. There is no doubt that these specimens came from genuine cases of Scabies Norvegica. Beatty has figured two cases (1913, 1915) and I have examined mites from each of these. I find that adults may be separated from those of typical *S. scabiei* var. *hominis* on one character only, and that is the greater size of the spines (Figs. 3 and 4). One must always exclude the fourth spine of the outer row from one's measurements because of its peculiar shape and proportions: the other spines measure 34 microns or more, and are also slightly thicker than those of *S. scabiei* var. *hominis*. The second spine of the inner row measures 42 and 45 microns in two females examined, the second in the outer row 36 and 42 microns. I can find no other points of difference whatever. The specimens at my disposal have been dried, and it is therefore impossible to measure length and breadth, but their size and proportion do not appear to differ from those of typical *S. scabiei*. As the only characters by which this mite may be distinguished require an oil-immersion lens and a micrometer for their proper appreciation it appears best to relegate it to varietal rank. It is unfortunate that this form was originally named *Sarcoptes scabiei crustosae*. The third name was not used in a varietal or subspecific sense, and there is no doubt that "*scabiei crustosae*" was regarded as a specific name. It is technically invalid, because it does not conform to the binomial principle. Later writers have regularized matters by writing *scabiei-crustosae* with a hyphen, as an emendation. The name of this variety is therefore *Sarcoptes scabiei* var. *scabiei-crustosae* (Fürst.).

3. *Norwegian crusted scabies.*

Dr Wallace Beatty's specimens are interesting in another way: the preparations, which are made in Farrant's medium, are full of a species of *Aspergillus*. That the mould is not a contamination is proved by the fact that the conidiophores of the mould are to be found in all parts of the preparation. If

the mould had invaded the preparation after it was made conidiophores would be found only at the edge of the cover glass. I can find no previous reference to the occurrence of *Aspergillus* in the crusts formed in this disease, and one or two leading dermatologists whom I have consulted inform me that they are not aware that it has been previously recorded. We may be dealing with an accidental infection of the crust by a common saprophytic mould. In support of this Dr Aldo Castellani informs me that *Aspergillus* is extremely common in old scrapings of a number of skin diseases. On account of this it was believed for many years that the causative agent of *Tinea imbricata* was an *Aspergillus*. We must not dismiss altogether the possibility that Norwegian crusted scabies is a condition produced by a secondary infection of a case of ordinary itch with *Aspergillus*. Writers of most modern text-books of dermatology (Hyde and Montgomery, Hartzell, Sutton, Sequeira), regard the crusted variety as common scabies occurring in a dirty and negligent individual. I am inclined to agree with them and to explain the minute differences between the common *Sarcoptes scabiei* and *S. scabiei* var. *scabiei-crustosae* as adaptations of the variety to its environment. Incrustations, similar to those of crusted scabies in man, are common on mangy horses; but I am unable to find any difference between mites from a horse which had been covered with crusts for many weeks, and mites from milder cases.

The view is also held that *S. scabiei* var. *hominis* and *S. scabiei* var. *scabiei-crustosae* are distinct organisms producing essentially different diseases: this was the view held by Fürstenberg and by Canestrini and Kramer. Against this view one may urge the minuteness of the morphological differences between the species and the variety. Beatty's second case (1915) was believed to have been infected from a case of common scabies. Moreover Norwegian crusted scabies is such a rare condition that were its causative mite really distinct from *S. scabiei* var. *hominis* it would long ago have become extinct. Norwegian crusted scabies is a rare disease. It is much to be desired that some one who is fortunate enough to see a case should infect a few volunteers in order to discover whether ordinary itch or the crusted variety is induced, and whether, after one or two generations, the mites can in any way be distinguished from typical *S. scabiei* var. *hominis*. A search should also be made for moulds.

A third view has been held, that Norwegian crusted scabies is due to an infection of man with a *Sarcoptes* derived from some other animal: Mégnin attributed it to *S. lupi*. The *Sarcoptes* of several of the common domestic animals (notably *S. scabiei* var. *equi*) produces in man a disease less, and not more, severe than ordinary human scabies.

An anomaly in chaetotaxy.

Fig. 9 shows an abnormality in the setae of an ovigerous female of *Sarcoptes scabiei* var. *hominis* collected by J. E. M. Mellor from our laboratory assistant Mr Reid after he had been artificially infected from a military case of scabies. The anomaly occurs on the left side only and consists in the reduplication of

seta *D* 2. The normal *D* 2 is present on both sides, and in its normal position: lateral to it on the left side is a seta one half of its length, and much finer. This anomaly is perhaps worth recording as the great setae are believed to be of considerable systematic importance.

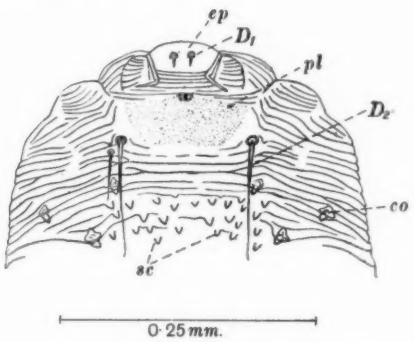


Fig. 9. *Sarcoptes scabiei* var. *hominis*, adult ♀. Part of dorsal surface showing reduplication of seta *D* 2 on left side: capitulum and legs omitted. *co* cone, *D* 1 and *D* 2 dorsal setae, *ep* epistome, *pl* plastron, *sc* scales.

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NOTES ON *RICKETTSIA*[1].

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Introduction.

Among the recent advances in our knowledge of the aetiology of human diseases, one of the most interesting is the discovery of a new group of somewhat problematic organisms which have been grouped together under the generic name *Rickettsia*. Up to now their presence has been shown, more or less satisfactorily, to be associated with three types of human fevers, viz. typhus, trench fever and possibly Rocky Mountain spotted fever, and in the last few years a certain number of papers have appeared on this subject, the most comprehensive of which are those published by the British War Office Committee and the American Commission on trench fever. In addition it has been suggested that many other human diseases are caused by these organisms, but the evidence is so very unsatisfactory that it may be disregarded.

Up to the present the papers which have been published on *Rickettsia* are concerned mainly with experimental and clinical investigations. With the object, therefore, of attempting to throw some light on the nature of the organisms themselves, in spring, 1919, at the suggestion of Professor Nuttall, I took up the study of the *Rickettsia* bodies associated with trench fever.

The investigation eventually resolved itself into three main divisions. First, the examination of a series of lice from persons known not to have been exposed to trench fever or typhus, in order to determine whether or not they contained *Rickettsia*; secondly, observations on *R. quintana* obtained by feeding lice, known to be uninfected, on a patient suffering from trench fever; finally, the examination of a number of blood-sucking insects in order to see how widespread is the occurrence of infection with *Rickettsia*.

(1) Examination of Uninfected Lice.

One of the arguments which has been used against the view that *Rickettsia* are parasites is the statement that these bodies may be found in uninfected lice and are normal constituents of the alimentary canal. Thus Brumpt (1918),

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as the result of an examination of lice collected from prisoners of war at Rennes, found *Rickettsia* in 53 out of 72 body-lice, whilst 27 head-lice were examined with negative results. Da Rocha Lima (1917) found similar organisms in lice collected from persons in Volhynia, but he remarks that they only occurred very sparingly and were confined to the lumen of the alimentary canal, only rarely invading the epithelial cells. To this organism he gave the name *Rickettsia pediculi*, and used the latter character to distinguish it from *R. prowazeki*, in which invasion of the epithelial cells is the usual occurrence. Various other Continental observers have also recorded the finding of *Rickettsia* in lice collected from persons who had not suffered from either typhus or trench fever. On the other hand Arkwright, Bacot and Duncan (1919), as the result of a careful examination of 245 specimens reared in the laboratory, found only one louse infected with *Rickettsia*.

Through the kindness of Mr Bacot I obtained a number of lice from the same stock that had been used in their experiments. These lice for many generations, extending back for more than three years, had been fed on a healthy person and had never produced any infection.

At the Quick Laboratory these lice were fed twice daily in the manner described by Nuttall (1917), on a laboratory attendant who had never been exposed to the possibility of infection with either typhus or trench fever.

When not in use the lice were kept in an incubator at 30° C. As a louse completes its life cycle in a period of less than a month, the stock used had been fed on healthy persons for at least forty generations. The faeces from these lice were examined at frequent intervals during a period of four months, but on only two occasions were any *Rickettsia* bodies found present. In addition the gut contents of 420 lice were examined by making smears, and staining with Giemsa in the usual way.

In the case of two individuals, bodies resembling *Rickettsia* were found, but the examination of the remainder was uniformly negative.

In addition 112 lice from various other sources were examined without finding any of these bodies.

The results of this examination of uninfected lice are in complete agreement with those obtained by Arkwright, Bacot and Duncan (1919), and by Wolbach, Todd and Palfrey (1920) in Poland. Only two naturally infected lice were found in 532, and therefore it is evident that this strain of lice is practically free from *Rickettsia* infection. The positive results obtained by Continental observers may be explained on two hypotheses.

(1) That owing to the prevalence of typhus and trench fever on the Continent during the war, the lice had become infected with *Rickettsia* by feeding on patients suffering from these diseases; and (2) that there is a species of *Rickettsia* which lives in the alimentary canal of the louse and is non-pathogenic to man.

Although the above-mentioned results would seem to favour the first hypothesis, it is difficult to explain all the positive results on the supposition

that the lice had fed on infected patients. In the first place Arkwright, Bacot and Duncan (1919), and the present writer found *Rickettsia* in three cases, and unquestionably these lice had never fed on any one infected with either trench fever or typhus. Moreover Töpfer (1916) and Da Rocha Lima (1917) were both well aware of the possibility of their lice having been contaminated and still came to the conclusion that there is a distinct parasite, *Rickettsia pediculi*, occurring in the human louse. The results of the examinations of other species of lice also confirm the view that there may be a *Rickettsia* specific to the body-louse.

(2) Observations on *Rickettsia quintana*.

Through the courtesy of Major-General Sir David Bruce, Chairman of the Trench Warfare Committee, a case of trench fever at the Mill End Hospital, Hampstead, was placed at my disposal. Unfortunately, this patient had only just recovered from an attack of roseola, but as his was the only case available, and he was suffering from an unmistakable attack of trench fever, it was decided to use him for obtaining *Rickettsia quintana*. Major Byam very kindly arranged for two boxes of lice from the Quick Laboratory to be fed daily on this patient for a period of 14 days. These lice were then examined by various methods and 14 out of 22 were found to be infected with *Rickettsia* in large numbers.

The faeces from both boxes, which previously had shown no sign of these organisms, were also examined and found to be infected. Although the amount of material was small, the appearance of the films made from infected lice was so strikingly different from any of the films made from uninfected lice that the presence of the *Rickettsia* was unmistakable. When seen singly or in very few numbers, it is possible to mistake them for staining artefacts, etc., but in the cases mentioned their definite shape and occurrence in clumps gave them a very characteristic appearance. However, as dried films stained with Giemsa are liable to produce artefacts, especially in the case of gut contents, some of the lice were fixed in various ways and afterwards sectioned and the sections stained.

The most satisfactory results were obtained by fixing in sublimate alcohol containing 5 per cent. acetic acid and afterwards staining by Giemsa's wet method, using acetone instead of alcohol for dehydrating. Other lice were fixed in Flemming's solution, Bouin and Gilson, respectively, and afterwards stained by different methods.

The aniline stains gave but indifferent results, as *Rickettsia* seem to have very little affinity for the ordinary staining reagents. When stained with iron haematoxylin it was very difficult to obtain any degree of differentiation that would clearly distinguish them from surrounding structures. When the sections were under-differentiated it was possible to recognise the outlines of the *Rickettsia*, but the organisms always appeared slightly smaller than in sections stained with Giemsa.

In its staining reactions *Rickettsia* shows a very close resemblance to Spirochaetes, as the cytoplasm seems to contain a relatively small amount of chromatinic substance which is diffused evenly through it. For demonstration purposes Giemsa or Leishman is by far the best stain to employ, as the colouring matter is precipitated round the surface of the organism, and therefore it appears somewhat larger and has a fairly distinct outline.

As noticed by previous observers, *Rickettsia* from the gut of the louse presents the appearance of round, oval, or diploid bodies, often occurring in clumps which are obviously the result of continued division without separation of the individuals. The parasite stains a purplish-blue colour with Giemsa and its dimensions may be estimated as about 0·3μ in diameter by 0·3–0·5μ in length. When dividing, the chromatinic material is concentrated at the opposite poles of the cell, thus giving the appearance of two darker stained bodies united by a somewhat lightly stained substance. Eventually the two halves separate, but as division seems to proceed rapidly these so-called diploid forms are usually present and form a characteristic feature.

The examination of sections and wet films has not resulted in finding any structure in *Rickettsia* which cannot be observed just as well in dried films, and as the latter are much simpler to prepare their use is recommended for this organism.

With regard to the occurrence of *R. quintana* in the alimentary canal of the louse the parasites are confined to the lumen of the gut and I have not observed their entry into the epithelial cells lining the alimentary canal or into any other parts of the body. The organisms seem to become attached to the surface of the epithelial cells of the midgut, and as they multiply gradually fill the lumen of the gut and extend backwards down the intestine, and eventually are voided together with the faeces.

The results of these observations confirm those obtained by Arkwright, Bacot and Duncan (1919) in their important series of experiments on the association of *Rickettsia* with trench fever. With regard to the systematic position of the organisms themselves, there is nothing to distinguish them from bacteria except their minute size.

The multiplication in the mid-gut and subsequent extension into other parts of the alimentary canal resembles that of *Bacillus pestis* in the gut of the rat flea. The insect host, in the case of *R. quintana*, merely furnishes a living culture tube in which the organisms grow and multiply without passing through any cyclical changes comparable with those that take place in the case of protozoa. The negative period of four days which intervenes between the ingestion of blood containing *Rickettsia* and the faeces of the louse becoming infective, is probably merely the result of the *Rickettsia* not being present in sufficient numbers to produce any infection, for it is well known in the case of other diseases that the inoculation of less than a minimum number of organisms does not produce any noticeable infection.

(3) Observations on other Species of *Rickettsia*.

Being unable to obtain additional cases of trench fever owing to its gradual disappearance after the war, a number of insects from various sources were examined in order to see whether other species of *Rickettsia* could be found.

Rickettsia melophagi.

Through the kindness of Messrs Cooper, Sons and Nephew, Watford, large numbers of *Melophagus ovinus* were obtained from sheep in various parts of the British Isles. The examination of these sheep-keds revealed the presence of *Rickettsia* in the alimentary canal of a large proportion of these insects. It seems to be fairly widespread for in addition to Germany, where it was noticed by Nöller (1917) and Sikora (1918), I have found it in *Melophagus* from various parts of England, Scotland and Ireland.

R. melophagi closely resembles *R. quintana*, both in its morphology and method of multiplication. The organisms are especially abundant in the middle and hinder region of the stomach, where they often form a layer on the surface of the epithelium. It is difficult to be certain that they do not penetrate into the cells themselves, but the appearance of sections suggests that they only occur on the surface in the same way as *R. quintana*. The organisms pass to the outside together with the faeces and presumably the infection is thus transmitted from one *Melophagus* to another.

According to Sikora (1918) *Rickettsia melophagi* is transmitted hereditarily to the offspring of infected *Melophagus*, in which case it is evident that the organisms must have the power of passing through the tissues and invading the ovaries or testes. The examination of sections of the developing ova resulted in finding a few bodies that might have been *Rickettsia*, but when present in small numbers it is difficult to distinguish them with any certainty from artefacts and therefore the results were inconclusive. However, the presence of *Rickettsia* in the young offspring of infected *Melophagus*, renders it probable that this infection is hereditary.

The *Rickettsia* were frequently associated with *Crithidia melophagi* and occasionally a parasitic nematode was also found in the alimentary canal of the *Melophagus*.

Rickettsia trichodectae n. sp.

The examination of Mallophaga from the horse resulted in the discovery of *Rickettsia* in 7 to 8 per cent. of the lice examined. The species of Mallophaga was *Trichodectes pilosus* and the presence of a *Rickettsia* in these insects is especially interesting as they do not feed on blood, and therefore cannot acquire the infection by the ingestion of infected blood.

The parasites occur in the alimentary canal of the *Trichodectes* and closely resemble *Rickettsia melophagi* in their morphology. Their dimensions are about 0·3–0·5μ in diameter by about 0·5 to 0·9μ in length and occasionally longer

forms may be observed. The organism multiplies in the alimentary canal and is passed out together with the faeces, thus furnishing a means for the spread of the infection from one insect to another. The name *Rickettsia trichodectae* is provisionally given to this parasite, for in spite of the absence of any distinguishing morphological characters, it can hardly be doubted that it represents a new species, in view of its occurrence in a distinct host.

Rickettsia linognathi n. sp.

The examination of smears from the alimentary canal of the goat louse, *Linognathus stenopsis*, resulted in finding *Rickettsia* in two examples out of 57 collected from goats at Cambridge. The parasite does not differ morphologically from the preceding species and as it was only found in smears of the contents of the alimentary canal it has not yet been possible to decide whether it occurs only in the lumen of the gut, or in addition in the epithelial cells. For convenience of reference the name *R. linognathi* is given to this organism, although as in the case of many other bacteria it is impossible to give satisfactorily distinguishing morphological characters.

Examination of other Arthropoda.

In the course of this investigation the gut contents of many species of blood-sucking insects, lice, Mallophaga and fleas have been examined and it is noteworthy how remarkably free from bacteria is the alimentary canal of most of these insects. Occasionally rod-shaped bacilli and sarcina like bodies were found, especially in the films made from lice, but as a rule they were absent.

In the case of *Haemotopinus suis* from the pig, most of the films were found to contain irregular-shaped bodies resembling the symbiotic fungi which occur in various species of insects, and especially in Hemiptera (Buchner, 1912).

Discussion of results.

Up to the present time nine species of *Rickettsia* have been recorded in addition to *Dermacentroxenus rickettsia* Wolbach (1918) which probably belongs to the same group of organisms. These different species and their habitats are summarised in the following table:

Rickettsia prowazeki da Rocha Lima 1916. Occurs in the lumen of the intestine and the epithelium of the mid-gut of *Pediculus humanus* and in the blood and organs of typhus patients.

R. quintana da Rocha Lima 1916. Occurs in the lumen of the alimentary canal of *Pediculus humanus* and in the blood and organs of trench fever patients.

R. pediculi da Rocha Lima 1917. Occurs in the lumen of the alimentary canal of *Pediculus humanus*.

R. melophagi Nöller 1917. Occurs in the lumen of the alimentary canal and the ovaries of *Melophagus ovinus*.

R. ctenocephali Sikora 1918. Occurs in the alimentary canal of *Ctenocephalus canis*.

R. trichodectae n. sp. Occurs in the alimentary canal of *Trichodectes pilosus.*

R. linognathi n. sp. Occurs in the alimentary canal of *Linognathus stenopsis.*

Rickettsia sp. Sikora 1918. Occurring in the alimentary canal of *Psokus* and *Ctenopsylla musculi*, respectively.

All the *Rickettsia* mentioned in the above list inhabit the alimentary canal of Arthropods, and in addition two of them also occur in the blood of man and are associated with human diseases.

The examination of their hosts shows that these organisms have been found not only in blood-sucking insects, but also in at least two species that do not feed on blood, viz. *Trichodectes* and *Psokus*.

In view of these facts, therefore, it seems probable that the *Rickettsia* represent a group of micro-organisms which primarily inhabit the alimentary canal of insects and other Arthropods. Some of them live saprophytically in the mid-gut, whilst others, such as *R. prowazeki*, have become true parasites and invade the tissues of their host.

As they multiply in the intestine, numbers of the *Rickettsia* would be continually passing to the exterior in the faeces and doubtless this is the way in which the infection is usually transmitted from one host to another in the absence of any intermediate vertebrate host. In the case of blood-sucking insects such as lice, any organisms that are voided are continually being introduced into the blood of the host either through the open wound caused by the bite of the insect itself, or through excoriations of the surface of the body from independent causes. Under these conditions therefore any such organisms would have the chance of becoming adapted to a parasitic mode of life in the vertebrate host.

With regard to the difficult question of the relation between *R. quintana* and *R. pediculi* it seems not unreasonable to assume that here we have an example of a *Rickettsia* normally inhabiting the intestine of the louse which is becoming adapted to a parasitic mode of existence in the blood of man.

The presence of *Rickettsia* in apparently normal lice has been recorded by many competent observers and their results are not satisfactorily explained by the assumption that in all these cases the lice have come either directly or indirectly from patients suffering from typhus or trench fever. It is more in unison with the results to accept da Rocha Lima and Töpfer's hypothesis that there is a species of *Rickettsia*, *R. pediculi*, which lives in the alimentary canal of the human louse and is non-pathogenic to man. On the other hand, the results of Arkwright, Bacot and Duncan (1918) as well as my own, have conclusively proved that some strains of lice are practically free from *Rickettsia*, but when fed on trench fever patients become infected with *R. quintana*, an organism that seems to be indistinguishable from *R. pediculi* except with regard to its physiological character of living in the blood of man.

To explain these results it is suggested that the human louse is liable to be infected with a non-pathogenic variety of *Rickettsia*, *R. pediculi*, which, under certain conditions, has acquired the capacity of living in the blood of man and

produced a pathogenic variety, for which it is convenient to retain the specific name *R. quintana*.

An interesting parallel may be observed in the case of parasitic flagellates. It is well known that these occur in the alimentary canal of a large variety of invertebrates and some species such as *Trypanosoma* and *Leishmania* have become adapted to living in the blood of vertebrates. Most of these flagellates inhabit the alimentary canal of blood-sucking insects, but, as in the case of *Rickettsia*, some are found in animals that never feed on blood and therefore cannot acquire the infection in this way. On the other hand the results of Laveran and Franchini (1914) and Fantham and Porter (1916) have shown that it is possible to infect vertebrates artificially by the inoculation of flagellates from various species of invertebrates, and there is no reason to suppose that the introduction of parasites into the body by natural methods would be any less successful.

In conclusion, I should like to express heartiest thanks to my friend Prof. G. H. F. Nuttall, F.R.S., for his continual assistance and helpful advice.

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THE ANATOMY AND BIOLOGY OF THE PARASITIC *APHELENCHI*.

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(With Figs. 1, 2 on Pl. VIII and Figs. 3–32 in Text.)

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I. THE PARASITIC SPECIES OF THE GENUS *APHELENCHUS* AND THEIR DISCOVERY.

A. fragariae Ritzema Bos, 1891.

A. ormerodis R.B., 1891. A doubtful species, possibly the young of *A. fragariae* (Marcinowski, 1908).

A. olesistus R.B., 1893.

A. phyllophagus n. sp.

Discovery of the Parasite.

Aphelenchus fragariae was first described by Ritzema Bos (1891) as causing a disease among strawberry plants in Kent; the plants had been sent to him by Miss Ormerod.

Nematoid worms were observed to cause disease in the leaves of *Begonia* by Worthington Smith (1890), in plants which came from Dunstable. In the following year Klebahn (1891) found *Aphelenchi* in blotched leaves of two species of fern (*Asplenium diversifolium* and *bulbiferum*) growing in a nursery in Bremen, and sent specimens to Ritzema Bos. The latter authority, having found the same species in these specimens and in *Begonia* leaves from England, described it under the name of *A. olesistus*.

II. DISTRIBUTION OF THE PARASITES, AND THE DISEASES CAUSED BY THEM.

A. fragariae. The disease caused by this species has been named strawberry bunch by Cobb (1891) (Blumenkohlkrankheit der Erdbeerpflanze, Ritzema Bos, 1891). It is characterised by stunting of growth in the length of the stem, and hypertrophy in breadth; the flowers abort, and fruit is not formed. Complete failure of the crop consequently results. It has been recorded from England, Scotland, Germany, and Norway (Marcinowski, 1908), and is doubtless much more widely spread.

A. olesistus since its discovery in *Asplenium* and *Begonia* has been found in many species of ferns and flowering plants. It attacks the leaves, causing large, sharply defined, brown blotches; the leaves finally wilt and fall off. Louis Mangin (1895) recorded the disease "de la Rouille" in Everlasting (*Helichrysum*) cultivated in the districts of Ollioules, Bandol, and St Nazaire, near Toulon. It causes great loss, as affected plants are unsaleable. A similar disease has been recorded from chrysanthemums by Atkinson (1891), from the United States by Sorauer (1901), Hofer (1901), Osterwalder (1904), and Molz (1909) from Switzerland and Germany. Ritzema Bos identified Sorauer and Hofer's nematode as *A. olesistus*. Sorauer (1902) described a serious epidemic in *Begonia* "Gloire de Lorraine," in which the plants were unsaleable. Osterwalder (1902) found the disease in flowering plants cultivated in the open air in Wadenswil and Zürich, in *Anemone japonica* and *Sylvestris*, *Ranunculus montana*, *Atragena alpina*, *Eryngium alpinum*, *Scabiosa sileni folia*, *Spiraea astilboides*, *Epipactis palustris* and others. It also occurs in *Coleus* and *Salvia* (Hofer, 1901) and in orchids (Marcinowski, 1908). Among ferns *Pteris ouvardi* and *cretica* have been found affected in addition to *Asplenium*.

Material used in the present investigation.

I received (1) specimens of strawberry plants containing *A. fragariae* from a correspondent in Ayrshire, (2) leaves of *Lygodium dichotomum* and *Lomaria ciliata* containing *A. olesistus* from the Royal Botanic Gardens, Kew,

through the kindness of Sir David Prain and Mr Arthur Hill, and (3) *Chrysanthemum* leaves containing the new species *A. phyllophagus*, from Messrs Tacon and Horwood, Cheshunt, Hertfordshire.

Regarding this disease in chrysanthemums, Messrs Tacon and Horwood write, that they have had this trouble for ten years at least; in one batch of pot chrysanthemums (Cheshunt White) one half were affected; they have been compelled to abandon certain varieties entirely, as they used to lose all their leaves.

Strawberry bunch and *Aphelenchus* leaf disease are therefore two widespread and destructive plant diseases, which cause considerable loss to the growers of fruit and flowers.

III. SPECIFIC DISTINCTNESS OF *APHELENCHUS FRAGARIAE* R.B., *A. OLESISTUS* R.B., AND *A. PHYLLOPHAGUS* N.SP.

A. fragariae and *olesistus*. Ritzema Bos distinguished his two species by their pathological effects on their hosts. Several points of anatomical distinction must have been observed under low powers of the microscope, but they are unsatisfactory. The tail of *A. fragariae* narrows suddenly behind the anus, that of *A. olesistus* does not.

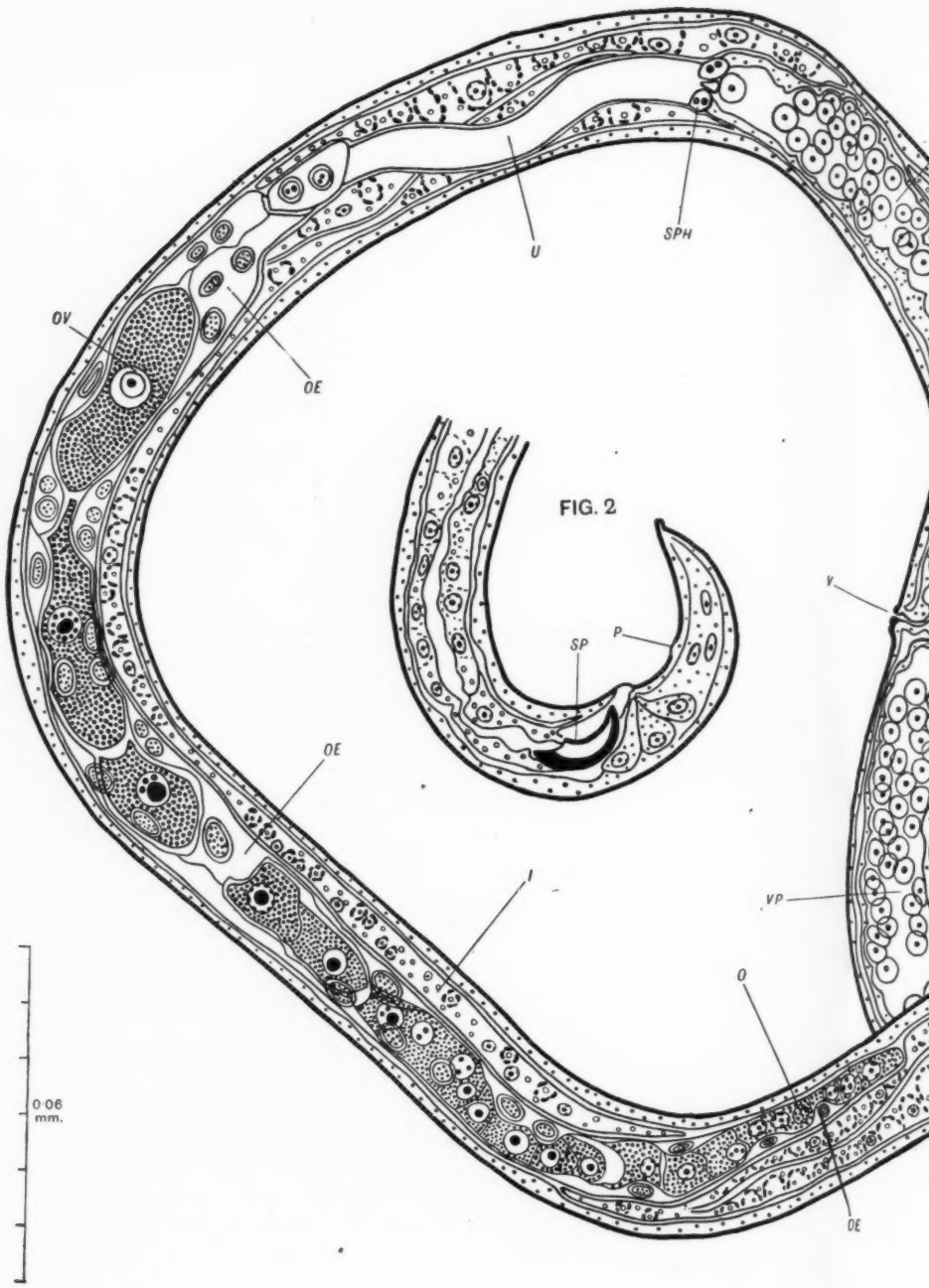
Marcinowski (1908) considers *A. fragariae* identical with *A. olesistus*, and possibly also with *A. helophilus* De Man. The differences in measurements she considers to be within the limits of individual variability. She performed experiments which she claims prove that the *Aphelenchi* of orchids and begonias can be transferred to strawberry plants.

> Infected orchid leaves were placed in contact with (1) begonias, (2) strawberry plants. In (1) immediate infection occurred with typical leaf disease; in (2) on the other hand no *Aphelenchi* were found on the strawberry plants during the first month, thereafter only a few in one plant; leaf disease and not characteristic strawberry bunch resulted.

This experiment therefore so far from proving the identity of *A. olesistus* and *fragariae* tends to prove their distinctness.

A. phyllophagus. When numerous fresh and well-mounted specimens are compared with *A. fragariae* and *olesistus* the following differences are obvious: it is a larger and more robust animal, and the excretory pore is situated some distance behind the nerve ring, while in *A. fragariae* and *olesistus* it is on a level with the nerve ring.

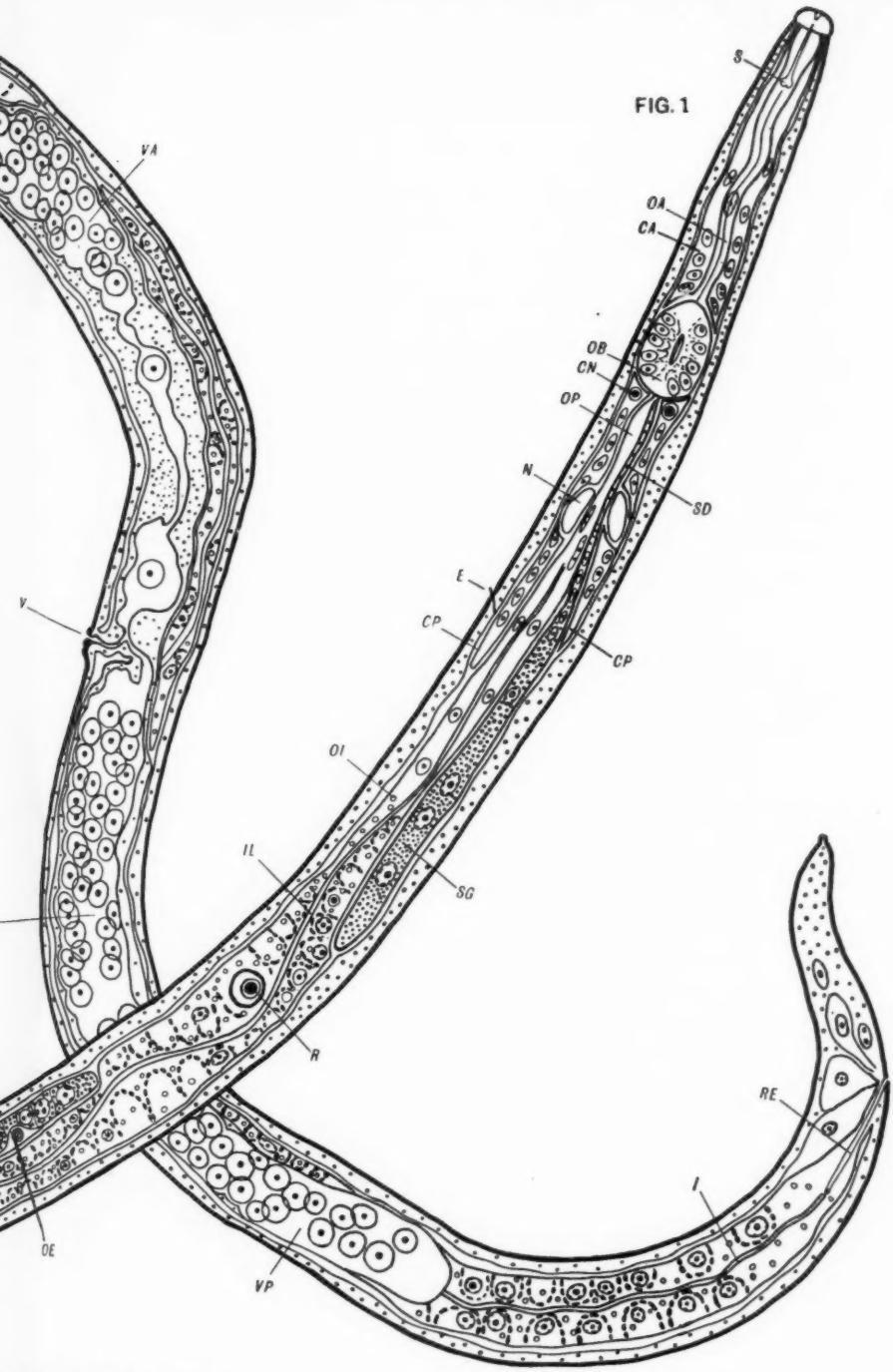
The difference in size between *A. phyllophagus* and *A. olesistus* is not a mere temporary difference between individuals of the same species due to the occupation of different host plants. I transferred both species to cabbage seedlings, but they retained their differences of size in the new host. It should be noted that the stomata (through which the parasites enter the plants, *vide* p. 176, E) are larger in the normal hosts of *A. phyllophagus* (chrysanthemums) than in the normal hosts of *A. olesistus* (begonias and ferns). The two species are therefore adapted to their normal hosts.



Figs. 1 and 2. **Aphelenchus phyllo**

Fig. 1. Adult female from a chrysanthemum leaf; outlines drawn with a camera from a specimen fixed with 7
stained with haemalum, and mounted in balsam.

FIG. 1



lenchus phyllophagus n.sp.

imen fixed with 70 per cent. alcohol, and mounted in glycerine jelly, detail completed from specimens d in balsam. Fig. 2. Tail of male.

KEY TO THE GROUP OF THE SLENDER-BODIED *APHELENCHI*.

Aphelenchi of slender-body form. a[1], 45 and over.

(1) Excretory pore behind the nerve ring. Parasitic. L. 0·85–1 mm. *A. phyllophagus* n.sp.

(2) Excretory pore at the level of the nerve ring.
  (*a*) Intra-uterine egg rounded oval. Free living. L. 1 mm. *A. helophilus* De Man, 1886.
  (*b*) Intra-uterine egg elongated cylindrical. Parasitic.
    (i) Anterior lip of anus prominent. Causes hypertrophy of tissues of host. L. 0·07–0·08 mm. *A. fragariae* R.B., 1891.
    (ii) Anterior lip of anus not prominent. Does not cause hypertrophy. L. 0·05–0·06 mm. *A. olesistus* R.B., 1893.

IV. *A. PHYLLOPHAGUS* N.SP. ANATOMY OF THE ADULT.

Measurements. ♀, L. 0·845–0·92 mm.; a, 51–53 (β, 11·3–16·5)[2]; γ, 20·5–21. ♂, L. 0·88–0·96 mm.; a, 48–52 (β, 12·6–13); γ, 16–19.

Cobb's formula:

$$\text{♀}\ \frac{1\cdot3\ \ (6\cdot8)\ \ 9\ \ ?\ \ 70\ \ 95\cdot5}{1\cdot2\ \ (1\cdot6)\ \ 1\cdot8\ \ ?\ \ 2\cdot1\ \ 1\cdot3} = 0\cdot923;$$

$$\text{♂}\ \frac{1\cdot2\ \ (7\cdot4)\ \ 9\cdot9\ \ ?\ \ M\ \ 93}{0\cdot8\ \ (1\cdot4)\ \ 1\cdot5\ \ ?\ \ 2\ \ 1\cdot5} = 0\cdot965.$$

Body-form (Figs. 1 and 2). The hemispherical *head* is marked off by a groove. *Lateral membranes* absent. *Transverse striae* of cuticle 0·0008 mm. in breadth. *Tail* ends in a mamilliform appendage. *Lateral lines* (Figs. 4–14, *LL*) occupy $\frac{1}{6}$th of the circumference. *Muscle fields* each contain five cells in cross-section.

Alimentary system. The *spear* (Fig. 1, *S*), length 0·013 mm., thickens slightly to the knobbed base. The *oesophagus* is divided into anterior oesophagus, bulb, and posterior oesophagus (Fig. 1, *OA*, *OB*, *OP*); the bulb is conoid and muscular; the intrinsic nuclei form a layer in its surface; the centre is occupied by the usual oval chitinous structure; the posterior oesophagus[3] is swollen slightly behind the bulb, narrows as it traverses the nerve ring, then increases gradually in width to its junction with the intestine, which is marked only by the commencement of the intestinal droplets and granules (Fig. 2, *OI*); the wall of the oesophagus is eosinophil in staining reaction, its lumen circular in the posterior section. The *salivary glands*[3] (Figs. 1, 11 and 12, *S.G.*) lie dorsal to, and slightly to the right of the posterior oesophagus and the commencement of the intestine; they consist of four cells arranged in linear series: the duct (Figs. 1 and 6–10, *S.D.*), containing several nuclei, passes forward through the nerve ring, and enters the oesophageal bulb at its posterior end (Fig. 5, *S.O.*); both gland and duct are basophil.

[1] De Man (1886) employs the following useful contractions:

$$\frac{\text{Length}}{\text{Breadth}}\ a. \qquad \frac{\text{Length}}{\text{L. of oesophagus}}\ \beta. \qquad \frac{\text{Length}}{\text{L. of tail}}\ \gamma.$$

Length, L. Maximum breadth, B.

[2] The length of the oesophagus is measured throughout to the posterior end of the bulb, owing to the indefiniteness of the function of the oesophagus and intestine. This measurement is given as the second figure in Cobb's formula—in brackets. The formula therefore runs:

Posterior end of spear, (Posterior end of bulb), Nerve ring, ?, Vulva, Anus.

[3] Cf. p. 169 (1).

The *intestine* (Figs. 1, 13 and 14, *I*) is thick and patent; walls without cell limits but containing numerous nuclei, droplets and granules; staining reaction basophil; the lumen is sinuous, flattened and slit-like, contrasting with the round lumen of the posterior oesophagus. The *rectum* (Fig. 1, *RE*) is one half to two-thirds as long as the tail.

Nerve ring and oesophageal collar. The *nerve ring* (Figs. 1, 9 and 10, *N*) is situated behind the oesophageal bulb at a distance rather greater than the length of the bulb. The *oesophageal cellular collar* (Figs. 1 and 6–12, *CA, C.P.*) is an ingrowth from the four longitudinal lines (Fig. 8, *C.P.*) and clothes the oesophagus throughout its whole length; it contains many nuclei, of which two or four situated immediately behind the bulb (Figs. 1 and 6, *CN*) are particularly large and prominent; the function of the collar is probably nervous, it, together with the nerve ring, representing the central nervous system of the animal.

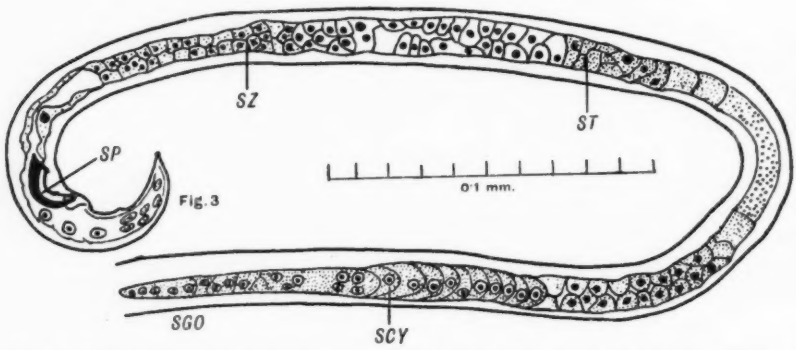
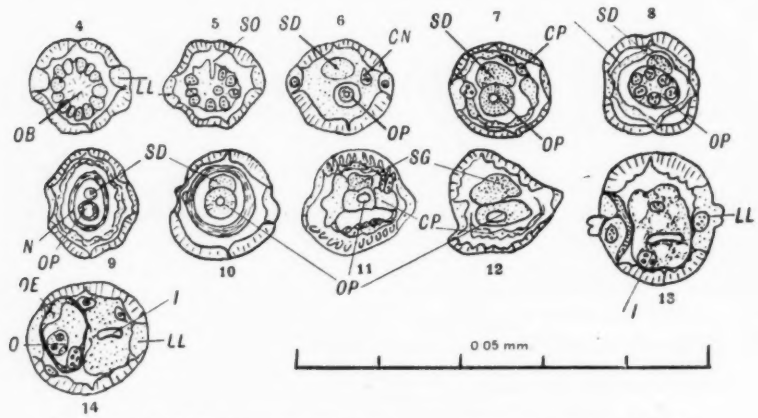


Fig. 3. **Aphelenchus phyllophagus** n. sp. Posterior two-thirds of male, showing gonads.



Figs. 4–14. **Aphelenchus phyllophagus** n. sp. Transverse sections in series.

Figs. 4, 5. Through the oesophageal bulb.
Figs. 6–8. Between the bulb and the nerve ring.
Figs. 9, 10. Through the nerve ring.
Figs. 11, 12. Between the nerve ring and the end of the oesophagus.
Fig. 13. Through the intestine in front of the gonad.
Fig. 14. At the commencement of the ovary.

Excretory system. The single *renette*[1] cell (Fig. 1, *R*) lies between the left lateral line and the intestine, at a level midway between the end of the salivary gland and the commencement of the gonad; it is somewhat difficult to recognise; in unstained preparations it has the appearance of a spherical space containing a small highly refringent sphere, which in stained specimens proves to be a chromatin mass. I have not succeeded in tracing the duct between the renette cell and the excretory pore; this aperture is situated behind the posterior margin of the nerve ring at a distance varying slightly in different specimens, but averaging 0·02 mm., equal to rather more than the length of the oesophageal bulb; this position markedly behind the nerve ring is of systematic importance (*vide supra*); the excretory duct contains a substance which stains with haematoxylin, giving a curious appearance, as if a spine were projecting through the cuticle.

There are no *caudal glands.*

The reproductive system in the female consists of two tubular sacs (Figs. 1 and 14, *OE, VA, VP*) extending forward and backward from the vulva; the anterior end of the anterior sac contains the ovary (Figs. 1 and 14, *O*); the ova (Fig. 1, *OV*), as they pass backward increase in size and become elongated oval in shape; the wall of this part of the sac consists of a fairly robust endothelium, which, in those places where it contains nuclei, bulges into the lumen and compresses or separates the ova (Fig. 1, *OE*); the nuclei have a characteristic circumvallate appearance. The structure of the *uterus* (Fig. 1, *U*) is difficult to analyse; it is closed at its posterior end where it joins the anterior vagina by a sphincter (Fig. 1, *SPH*). It is probable that fertilisation and formation of the shell occur in the *anterior vagina.* Marcinowski (1910) describes a shell gland in *Cephalobus,* but it should be remembered that Schneider (1866, p. 285) proved that the shell of nematode eggs is secreted by the egg itself, and not by the wall of the oviduct. The anterior vagina extends from the sphincter to the vulva; its walls are of irregular thickness, and do not contain the typical nuclei of the ovarian sac; the lumen is occupied by a mass of spherical spermatozoa. The retrovulvar portion of the reproductive tube consists of the *posterior vagina* alone, which resembles the anterior vagina in length and other respects, but is a caecum; the vaginae function as receptacula seminis. (For development in the larva, and early fertilisation, see Sect. V below: for comparative anatomy, p. 172 (3).) The *vulva* is a transverse slit situated $\frac{7}{10}$ths of the body-length from the head.

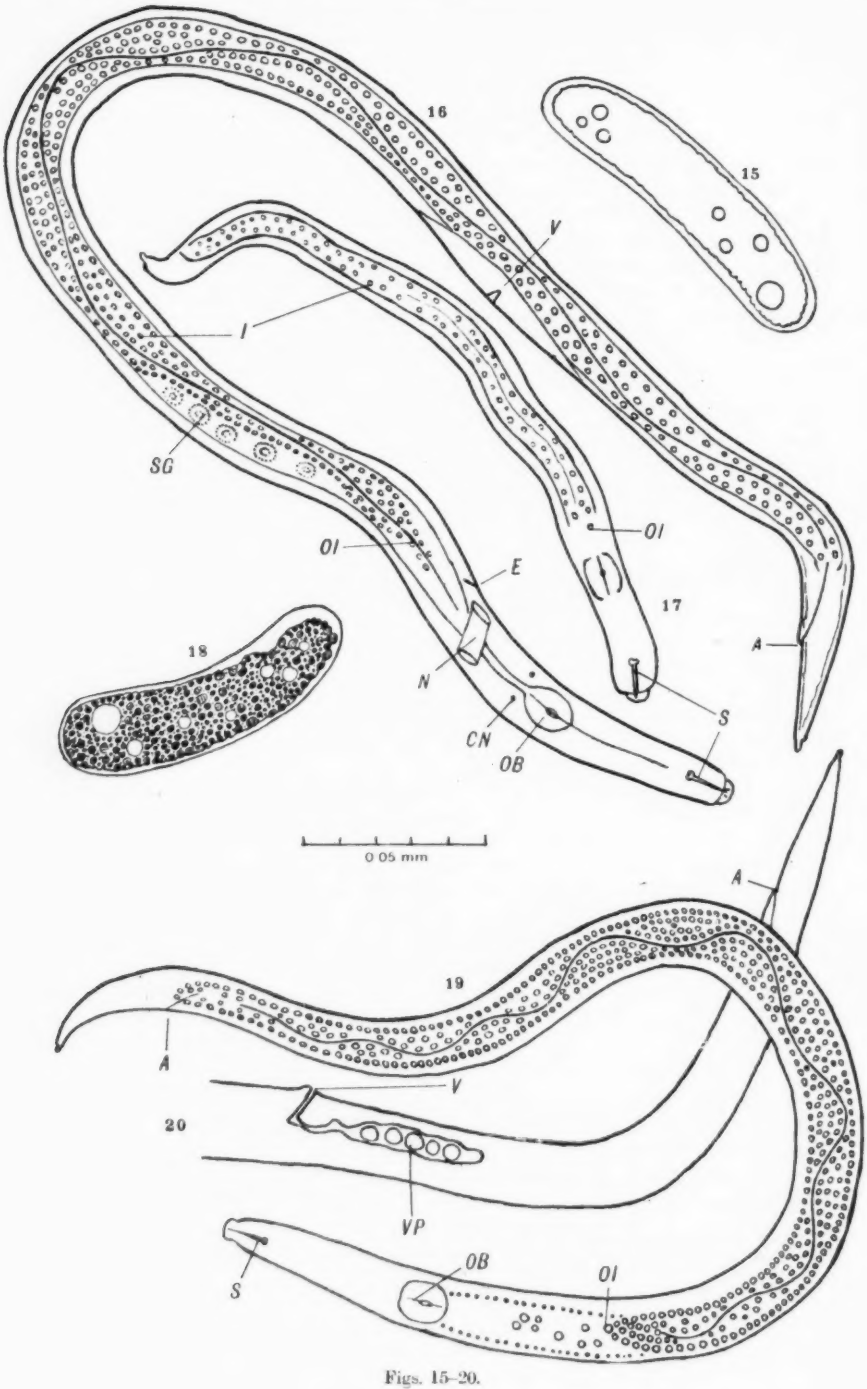
Male reproductive organs (Figs. 2 and 3). The single testicular tube has an endothelial wall, like the ovarian; posteriorly it joins the intestine to form the cloaca, and is here closed by a valve-like projection of the ventral wall. The *spicules* (Figs. 2 and 3, *SP*) are very broad, curved and hollow, the posterior margin is much thickened, the anterior margin is the structure described and figured by Ritzema Bos (1893) as the accessory piece; the two spicules are very closely apposed; there is no accessory piece. The *tail of the male* (Fig. 2) is sharply curved, the ventral surface flat, with one poorly-defined papilla, post-anal, median ventral (Fig. 2, *P*); in mounted specimens there is sometimes an appearance of a bursal membrane, which is probably artificial, but the flattening of the ventral surface must produce a ridge along each lateral line which forms the rudiment of a bursal membrane.

V. *A. PHYLLOPHAGUS.* THE EGG, AND LARVAL DEVELOPMENT.

The *egg* (Figs. 15 and 18) is sausage-shaped, 0·085 × 0·023 to 0·095 × 0·022 mm. The shell is thin and unsculptured, the ovum when first laid unsegmented. I have not observed segmentation or embryonic development.

Larvae. Molz (1909) gives the length of the newly-hatched larva as 0·16–0·18 mm. and its breadth 0·01 mm. (*a* 16–18). The youngest larva which I have seen (Fig. 17) measured 0·22 × 0·015 mm. (*a* 14·7), the spear 0·012 mm.; it was found in the leaf axil of a groundsel on the 28th day after infection, together with adults and eggs, and had recently hatched

[1] Cf. p. 172 (2).



Figs. 15–20.

in that situation. An older larva (Fig. 19) measured 0·465 × 0·02 mm. (*a* 23), spear again 0·012 mm.; it was found in the tissue of a leaf of a groundsel on the 5th day after infection, together with larvae measuring 0·25, 0·3, and 0·32 mm. Being thus associated with recently hatched larvae it was probably derived from an egg laid within the previous five days in a leaf axil of the plant. In both the 0·22 mm. and the 0·465 mm. larva the oesophageal bulb was distinct; the intestine (Figs. 17 and 19, *OS*) commenced behind the bulb at a distance equalling head to bulb; the alimentary canal was patent.

A still older larva (Fig. 16) measured 0·65 × 0·018 mm. (*a* 36); spear still 0·012 mm.; the *salivary gland* (*SG*) was apparent, with five nuclei of which the anterior probably forms the duct; the gland was situated further back than in the adult, the whole of it lying behind *OI*; the nerve ring (*N*), and the two large nuclei of the posterior collar (*CN*), were also apparent, the latter as two black dots which I at first took for eye spots; the excretory pore was in the same situation as in the adult. The rudimentary vulva was situated three-quarters of the body-length from the head; it lay in a clear area in the midventral line, which represented the rudiment of the vaginae. This specimen was found in a leaf of a groundsel, ten days after infection, together with adults and other larvae. A young female (Fig. 20), from the same leaf, was probably also hatched in the same situation, not more than ten days previously; it measured 0·8 × 0·022 mm. (*a* 36).

The outstanding fact in this specimen was the condition of the developing genital ducts, the vulva and the posterior vagina only being present, the latter already containing spermatozoa! The rudiment of the ovary was not observed, but there was no trace of germinal cells at the fundus of the vagina; a condition of protandrous hermaphroditism was therefore not present, such as occurs in *Rhabditis* (*Leptodera*) (Schneider, 1866, p. 316).

Length of time required for embryonic and larval development, and by one generation from egg to egg.

We have seen above that recently hatched larvae, measuring 0·25–0·46 mm., have been found in the leaf of a plant exposed to infection for only five days, older larvae, 0·65, and young adults after only ten days. We may therefore conclude that embryonic development does not occupy more than five days, complete embryonic and larval development not more than ten, and that a generation from egg to egg could be completed in fourteen days, the conditions of temperature being those of a European spring, summer or autumn, with the day temperature not falling below 15° C. (60° F.). This agrees with the observations of Ritzema Bos (1892) on *Tylenchus dipsaci* (*devastatrix*) Kühn. As in this species, and in contrast to *T. tritici*, many generations can thus succeed each other in the course of one year, enormous multiplication may occur in a short period.

Figs. 15, 16. **Aphelenchus phyllophagus** n. sp. Specimens from *Senecio vulgaris*.
Fig. 15. Egg from leaf axil, 28th day. Outline.
Fig. 16. Larva, 0·65 mm., from mesophyll, 10th day.
Fig. 17. Larva, 0·22 mm., from leaf axil, 28th day.
Fig. 18. Egg from leaf axil, 28th day.
Fig. 19. Larva, 0·465 mm., from mesophyll, 5th day.
Fig. 20. Young female, 0·8 mm., posterior third of body, from mesophyll, 13th day.

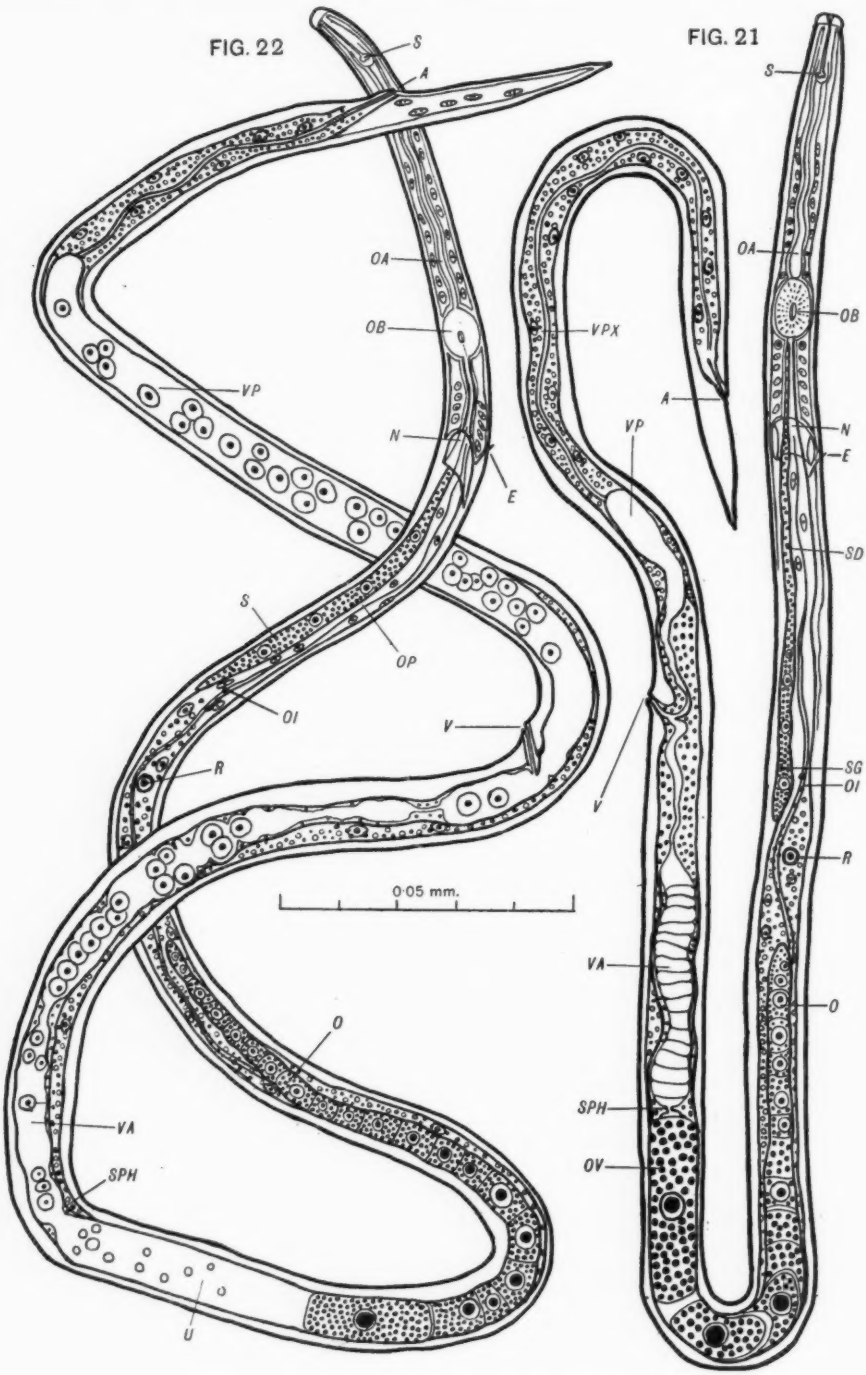


FIG. 22
S
A
OA
OB
VP
N
E
S
OP
OI
V
R
0·05 mm.
O
VA
SPH
U
FIG. 21
S
OA
OB
VPX
A
VP
N
E
SD
SG
OI
R
V
VA
O
SPH
OV

VI. *A. OLESISTUS* R.B. 1893. ANATOMY. (Figs. 21, 23, 24.)

Measurements. ♀ L. 0·529 mm., B. 0·011 mm., *a* 48, (β 8), γ 20. ♀ L. 0·574 mm., *a* 44, (β 11), γ 19. ♂ L. 0·5 mm., *a* 50, (β 8·87), γ 15.

Cobb's formula: ♀ $\frac{1{\cdot}8 \quad (9) \quad 11{\cdot}8 \quad ? \quad 70 \quad 95}{1 \quad (1{\cdot}8) \quad 2 \quad ? \quad 2{\cdot}3 \quad 1{\cdot}2}$ 0·574.

In general anatomy, this species, as well as *A. fragariae*, so strongly resembles *A. phyllophagus*, that it is necessary to refer to a few points only. *Cuticular striae* are not visible. *Spear*, 0·01 mm. *Excretory system*, the renette cell (*R*) lies in the same position on the left side; the excretory pore (*E*) is however at the level of the nerve ring. *Reproductive system*: *female*, the structure is naturally more difficult to distinguish than in the larger species; in the specimen figured the uterus is occupied by a large cylindrical egg without a shell; the sphincter at the junction of uterus and anterior vagina is well developed; the spermatozoa occupying the anterior vagina are arranged in a rouleau. (The posterior vagina extends further back than is shown in Fig. 21, to the point marked *VPX*.)

Male spicules (Fig. 24). Length, 0·013 mm.; no accessory piece, no caudal papilla.

VII. *A. FRAGARIAE* R.B. 1891. ANATOMY. (Figs. 22 and 25–29.)

Measurements. ♀ L. 0·723 mm., B. 0·012 mm., head to posterior margin of bulb, 0·065 mm.; tail, 0·038 mm., *a* 60, (β 11), γ 19.

Cobb's formula: ♀ $\frac{1{\cdot}6 \quad (9) \quad 11 \quad ? \quad 69 \quad 93}{0{\cdot}8 \quad (1) \quad 1{\cdot}1 \quad ? \quad 1{\cdot}6 \quad 1}$ 0·723.

This is the most slim of the three species, especially as regards the oesophageal region of the body. Compare breadth at the nerve ring, 1·1 per cent. with 2 per cent. in *A. olesistus*.

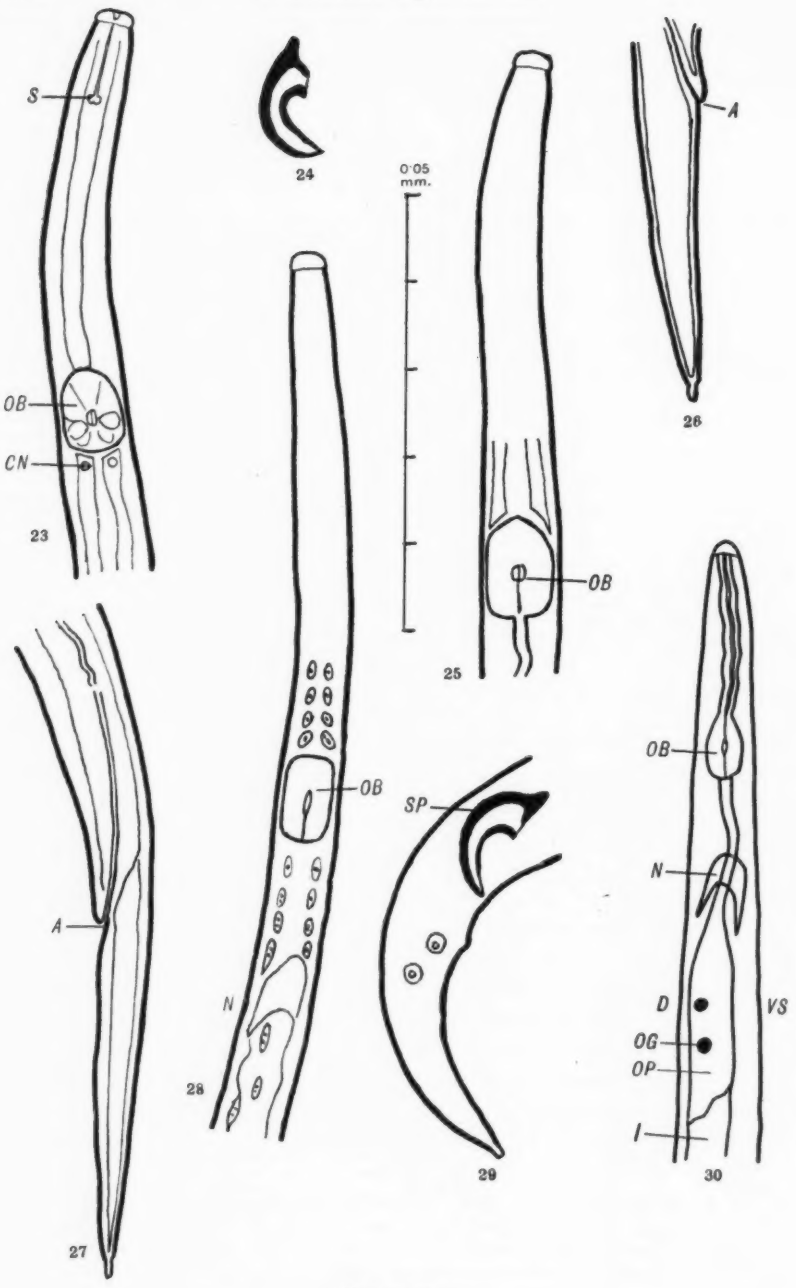
Spear, 0·01 mm. *Oesophageal bulb* conoid to cylindrical (Figs. 25, 26). *Rectum* (Fig. 27), long, nearly equal to the length of the tail. The anterior lip of the *anus* is prominent (Figs. 27, 28). *Excretory pore* at the level of the anterior margin of the nerve ring. *Female reproductive system*: the uterus is well-defined (Fig. 22, *U*), and is marked off from the anterior vagina by the sphincter (*SPH*); the anterior and posterior vaginae are of equal length. *Spicules* of the male (Fig. 29), 0·014 mm.; no accessory piece, the accessory of Ritzema Bos being again the anterior margin of the spicule.

VIII. COMPARATIVE ANATOMY OF (1) THE OESOPHAGUS AND SALIVARY GLANDS, (2) THE RENETTE, (3) THE VAGINAE, IN THE GENUS *APHELENCHUS*.

1. THE OESOPHAGUS AND SALIVARY GLANDS. In this genus the *oesophagus* is usually described as terminating at the bulb, and as joining the intestine at that point. The following facts, however, prove that the section of the alimentary canal between the bulb and the point of commencement of the intestinal droplets and granules (Figs. 1, 16–19, 21 and 22, *OI*) is morphologically the posterior oesophagus: (1) it is embraced by the nerve ring; (2) it is

Fig. 21. **A. olesistus** R.B., adult female from cabbage seedling infected 14 days previously from *Lomaria ciliata*. Fixed in Bouin's sol., mounted in glycerine jelly. (Note: The posterior vagina extends to the point *VPX*.)

Fig. 22. **A. fragariae** R.B., adult female from bud of [illegible] rawberry plant; fixed in Bouin's sol., stained with haemalum, and mounted in balsam.



Figs. 23–30.

clothed by the oesophageal cellular collar; (3) its walls are eosinophil in contrast to the basophil intestine; (4) its lumen is cylindrical, that of the intestine is flattened. The lack of a definite line of demarcation from the intestine is due to the separation of the oesophageal glands from the body of this portion of the oesophagus, which will be considered in the next paragraph.

The *salivary glands* have been described in *Tylenchus similis* Cobb, by that writer (1915). They resemble those of *Aphelenchus phyllophagus, olesistus,* and *fragariae.* (Cobb has, however, traced their duct through the substance of the bulb and anterior oesophagus to an opening at the base of the spear.) The posterior oesophagus in *Tylenchus similis* also resembles that of the *Aphelenchi,* and differs from the corresponding organ of other *Tylenchi, e.g. T. dipsaci* Kühn, in which it is thick, club-shaped, and glandular, and is sharply marked off from the intestine (Fig. 30). In *T. dipsaci* this posterior bulb contains several large nuclei in its dorsal wall, while no salivary glands are present. It is therefore a reasonable supposition that the posterior oesophageal bulb of the *Tylenchi* (less *T. similis*) represents, morphologically and physiologically, the combined posterior oesophagus and salivary glands of *Aphelenchus* and *T. similis*; in other words, the oesophageal glands of *Tylenchus,* which are situated in the dorsal sector of the posterior bulb, have, in *Aphelenchus* and *T. similis,* separated themselves from the oesophagus to form the salivary glands.

Considering further the anatomy of the oesophagus and its glands, in other nematode genera, we find three glands, one in each longitudinal sector of the organ, which open by three ducts into the alimentary canal (*Thoracostoma* and *Cylicolaimus* Jägerskiöld, 1901; *Oncholaimus* Stewart, 1906; *Ascaris,* adult, Jägerskiöld; *Agchylostoma* Looss, 1911). The dorsal gland is, however, always more important than the two subventrals, is longer, and stains more deeply (Jägerskiöld, 1901, p. 14). Finally on examining the larva of *Ascaris lumbricoides* (Stewart, 1921), we find that these three glands originate from a single giant nucleus in the hind end of the dorsal sector, which, as growth proceeds, expands downward into the subventral sectors. We therefore have in series (1) *Aphelenchus* and *T. similis* with the glands separate from the oesophagus, on its dorsal surface, as the "salivary glands"; (2) *Tylenchus* (less *T. similis*) and the larvae of *Ascaris* to the seventeenth day, with

Figs. 23, 24. **A. olesistus** R.B.

Fig. 23. Male, outline of oesophageal region.
Fig. 24. Male, spicule.

Figs. 25–30. **A. fragariae** R.B.

Fig. 25. Male, outline of oesophageal region.
Fig. 26. Female, outline of tail.
Fig. 27. Female, outline of tail.
Fig. 28. Female, outline of oesophageal region.
Fig. 29. Male, outline of tail.
Fig. 30. **Tylenchus dipsaci (devastatrix)** Kühn, from clover, fixed in 70 per cent. alcohol, mounted in glycerine jelly.

the glands in the dorsal sector; (3) *Ascaris* larva of the nineteenth day with glands growing from the dorsal sector into the two subventrals; (4) the great majority of adult nematodes with the typical three oesophageal glands.

2. The Renette lies between the left lateral line and the intestine in *Tylenchus similis* Cobb (1915), and the three species under consideration. In the development of *Ascaris lumbricoides*, it originates in the same position, and thence gives rise to the apparently bilateral organ of the adult Ascarids. This origin of the excretory cell from the left lateral line is striking, and may prove to be of very general occurrence. Without laying too much stress on the homology, it may be recalled that the great, unicellular, skin glands of both lateral lines are the excretory organs of *Cylicolaimus magnus* Villot, *Thoracostoma acuticaudatum* Jägerskiöld (Jägerskiöld, 1901), and of the adult female of *Oncholaimus vulgaris* Bast. (Stewart, 1906).

3. The Vaginae are derived, as we have seen (p. 167) from a superficial cell group, which gives rise to them only, and not to the remainder of the reproductive tube—the gonad proper. They are probably ectodermal, their walls are not endothelial, and their junction with the uterus is marked by a sphincter. The corresponding sphincter is situated at the junction of the vagina and uterus in *Cylicolaimus*, *Thoracostoma* (Jägerskiöld, 1901), and *Oncholaimus* (Stewart, 1906), in which genera the vaginae are much shorter than in *Aphelenchus*. In *Cylicolaimus* and *Thoracostoma* sperm is permitted to pass the sphincter, and the uteri function as receptacula; in *Aphelenchus* the vaginae are large enough to accommodate the whole sperm mass; in *Oncholaimus*, where sperm is not allowed to pass into the uterus, although the vagina is too small to contain it, the surplus is drained off into the intestine by the gonenteric canals (Stewart, 1906).

The posterior vagina is the only portion of the posterior reproductive tube (of *e.g. Tylenchus*) which persists in *Aphelenchus*.

IX. BIOLOGY OF THE PARASITIC *APHELENCHI*.

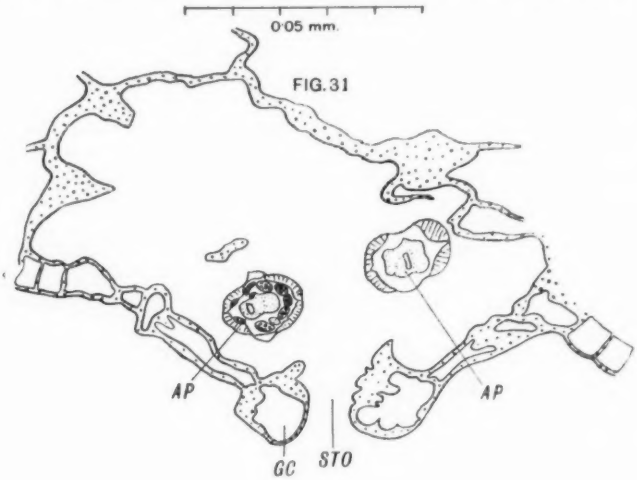
The following experiments and observations were made principally on the species *A. phyllophagus* and *olesistus*. It may, however, be assumed that the three species are indentical in their general mode of life.

A. Definitive habitat of the parasite.

Aphelenchus fragariae. The strawberry plants received by Ritzema Bos (1891) were not fresh, and he was therefore not able to make satisfactory observations on the situation of the parasite. Marcinowski (1908) found them to be mainly ectoparasitic, in the leaf axils, and in the flowers among the stamens, during the month of May; only a few were endoparasitic, in brown patches of leaf sheaths. In June, however, they were chiefly endoparasitic, in leaves and stem. She notes that apparently healthy runners contained *Aphelenchi* in the bud.

In specimens sent to me in January I found adult and larval *A. fragariae*, in large numbers as ectoparasites under the scales of the bud. In serial sections (Fig. 32), they are seen lying among the hairs on the surface of the growing point.

The definitive habitat of *A. olesistus* and *phyllophagus*, that in which feeding and reproduction proceed most actively, and in which their pathological effects are manifest, is in the mesophyll spaces of leaves; they are never



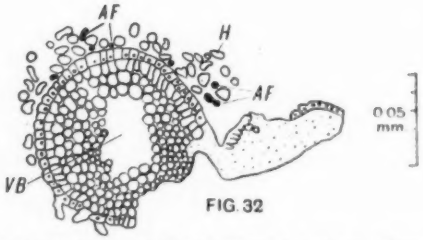


Fig. 31. Chrysanthemum leaf, transverse section through a stoma, showing **A. phyllophagus** in a mesophyll space.

Fig. 32. Strawberry plant, transverse section through the bud, showing **A. fragariae** on the surface.

intracellular, and avoid tissues without spaces (Ritzema Bos, 1893; Molz, 1909); they occur frequently close to the stomata.

MIGRATION IN THE TISSUES OF THE LEAF. Ritzema Bos (1893) believes that *A. olesistus* travels in or along the outside of the nerves. Molz (1909) denies this, and asserts that migration only occurs through the spaces of spongy tissue, and on the surface; he finds the vessels of chrysanthemum leaves to be too narrow to admit the parasite; the animal wanders out of the

diseased patches in search of fresh nourishment for itself and its progeny; eggs are found laid only in healthy tissue, consequently the gravid female has the greatest impulse to travel.

Adults and larvae *reach the soil* by the fall of the diseased leaves, bringing the life cycle of the race[1] to the resting stage in the soil.

B. The resting stage in the soil.

Marcinowski (1910) asserts that the parasitic *Aphelenchi* can live only for a very short period in the soil, in fact that they only enter the soil in order to pass directly from a fallen leaf to a new host plant.

She placed portions of infected leaves on the earth of a pot, which was kept damp, and examined the earth from the second week onward—result negative. On the other hand, earth in which an infected *Begonia* had grown, proved infectious when placed on leaves of begonias.

Ritzema Bos (1892) showed that *Tylenchus dipsaci* can persist in the soil only on the surface, where it can undergo partial drying, and so enter a dormant condition. I found that adult *A. phyllophagus* can live actively in water for at least six days. Early death in damp soil may be due to mycosis.

Molz (1909) apparently believes that *A. phyllophagus* resides in the soil.

I have made the following observations to test the power of survival in earth:

Portions of infected leaves of *Chrysanthemum* and *Lomaria ciliata* were placed on earth in pots, which were watered daily, the surface of which, however, remained dry except for a period not exceeding two minutes each day. On the *7th and 9th days* many adults, male and female, were found on the surface of the leaf fragments, a few actually in their tissues.

On the *16th, 17th, 20th* and *46th days the surface layer of earth* was examined. It was quite dry at the time, and had been at a temperature below the freezing point at night. Adult *Aphelenchi* were found in large numbers in a dormant condition, but they resumed activity after immersion in water. Larvae and eggs were looked for on the 46th day, but were not found. *In earth taken from* 10–15 *mm. below the surface* no *Aphelenchi* were found.

We can therefore conclude that *A. phyllophagus* and *olesistus* reaching the soil in fallen leaves, can live there for at least 46 days. Like *T. dipsaci*, they collect on the surface of the earth, where they suffer partial desiccation, and pass into a condition of suspended vitality. In the case of *T. dipsaci*, it is the larvae only which survive in this manner, while in *Aphelenchus* on the contrary the adults survive, and it is doubtful whether the larvae do so (they probably grow to adults before abandoning the fallen leaves).

[1] It should be clearly realised that the life cycle in *Aphelenchus* and *T. dipsaci* is that of the race, while in *T. tritici* it is that of the individual; in other words in the cycle from (*a*) the definitive habitat, through (*b*) the resting stage in the earth, and (*c*) the stage of immigration, many generations are completed in the former, only one generation in the latter; also many cycles can be completed in one year in the former, only one in the latter. Hence the enormously greater power of multiplication in the former than in the latter.

We now proceed to the consideration of:

C. THE ROUTE TAKEN BY THE PARASITE ON THE HOST DURING IMMIGRATION FROM THE SOIL.

There are two views on this subject: (1) that the parasite, having entered the host plant below the level of the ground, ascends through the tissues of the stem to the leaves (Ritzema Bos, 1893, and Marcinowski, 1908–1910); (2) that it enters through the surface of the leaf, having reached this situation by travelling over the surface of the plant.

(1) Ritzema Bos (1893) found *Aphelenchi* in apparently healthy leaf-stalks of *Begonia* and *Asplenium*. In an experiment with an infected *Pteris*, he cut off all the parts of the plant above ground; the young fronds which grew up contained *Aphelenchi*, and he concludes that they came from the interior of the rhizome; it is, however, equally probable that they came from the surface of the soil. Marcinowski (1908) found a few *Aphelenchi* in the stem of a plant. The two observations of *Aphelenchi* in the stem appear to have been made by dissection or teasing; in such a method it must be borne in mind, that worms lurking on the surface may appear in the medium, and it may be thought that they have issued from the tissues. I have found a few *Aphelenchi* on teasing up portions of flower stalk, but on cutting serial sections have never found them actually in the stalk or stem; in one case I divided a groundsel stem longitudinally in halves, one half was teased out in water, and numerous adults and larvae found, the other half was cut into serial sections, but no *Aphelenchi* were found in the tissues, although several appeared on the cut surface.

The following observations also are offered to this view: the parts of an infected plant below the leaves are healthy (Klebahn, 1891); plants placed among infected leaves, if protected by a ring of vaseline around the stem, remain free of the parasite (Marcinowski, 1908); if all diseased leaves are removed from a plant, the buds growing out of the axils remain free (Molz, 1909).

We can therefore conclude that *Aphelenchi*, in their invasion of the host plant, do not traverse the tissues of the stem.

APHELENCHI *in the soil, revived by moisture, and attracted by a suitable plant, wander on to it, and may live for some time as ectoparasites in the leaf axils before reaching their definitive habitat in the mesophyll.*

In order to trace the course of immigration, I placed a number of young plants of groundsel (*Senecio vulgaris*) in pots of earth, on the surface of which infected leaves of *Chrysanthemum* and *Lomaria* had been strewn. The pots were kept in a room at a temperature of 10–15° C. (50–60° F.), were freely exposed to the sun, and were watered once daily. Entire plants or leaves were examined at intervals with the following results:

2nd day. Four entire plants negative.

3rd day Three plants examined. Plant (*a*): on syringing out the leaf axils with water three adult males and four females were found; in a leaf touching the ground—one adult female. The earth in this case had been infected 44 days previously, and had been bare of vegetation for some hours before the planting of plant (*a*). Plant (*b*): in a leaf axil one adult, on a leaf one larva, 0·305 mm. long. The earth had been infected 43 days previously, a chrysanthemum which bore ectoparasitic *Aphelenchi* was growing in the same pot. Plant (*c*): negative.

5th day. Lower leaves from four plants. (*a*), (*b*) and (*c*) were negative. (*d*): in the tissues of one lower leaf four larvae found. 0·25, 0·3, 0·32, 0·465 mm. long; soil infected 45 days previously; the leaf axils were unfortunately not examined, but it is probable that they contained adults which were the source of these larvae, a chrysanthemum was however also growing in the pot. *10th day.* In a low growing leaf many adults and larvae found; the pot had been infected 41 days previously, and had been bare of vegetation for three days before the planting of this specimen, the adults must therefore have lived at least three days, and almost certainly 31 days in the soil before attacking this plant. *8th, 13th and 26th days.* Adult *Aphelenchi* in the leaf axils or lower leaves. *28th day.* Adults, larvae and eggs found in the leaf axils of the lower 20 mm. of the stem, adults and larvae also in the tissues of the lower leaves.

Marcinowski (1910) with begonias planted in infected soil, found that leaves in contact with the earth were invaded.

We can therefore conclude that adults invading a plant from the soil may take up ectoparasitic life in the leaf axils up to a height of at least 2 cm. above ground, and may here deposit eggs; the larvae hatched from them then proceed to the more extended invasion, and to their definitive habitat in the mesophyll. On the other hand, leaves close to the ground level may be invaded direct from the soil, the worms becoming entoparasites forthwith.

This brings us to the passage from ecto- to entoparasitic life, and the mode of entry into the spaces of the mesophyll:

D. *Aphelenchi* having reached the leaf surface enter the mesophyll spaces through the stomata.

This statement rests on the following observations: (1) *Aphelenchi* from orchid leaves, dropped in water on the ventral surface of leaves of an inverted *Begonia*, entered the leaves; application of infected to healthy leaves gave the same result (Marcinowski, 1908). (2) *Aphelenchi* have been observed traversing the stomata (Osterwalder, 1902 [quoted by Marcinowski], Marcinowski, 1908).

Molz (1909) held that the worms did not enter through the stomata, but through wounds of the plant surface, on the ground that (1) the stomata were too small to admit the parasites, and (2) leaf to leaf infection succeeded only with infected leaves. On the other hand, (1) my measurements of *Chrysanthemum* stomata give larger apertures (viz. 0·02 × 0·01 mm., and the shorter diameter can be increased by the forcible abduction of the guard cells), and the size of the parasite varies with the size of the stomata (*vide* bottom of p. 162); and (2) the negative result of Molz's leaf to leaf infection is explained by conditions of atmospheric humidity (Marcinowski, 1910, *vide infra* (E)).

E. Influence of atmospheric humidity, and of previous disease or injury of the host plant, in assisting migration of the parasite. Immunity.

Marcinowski (1908, 1910) records three important experiments on the influence of damp on the migration of *Aphelenchi*. (1) A portion of infected chrysanthemum leaf was mounted, ventral surface uppermost, in water on a slide; many worms were observed to creep out through the stomata. (2) If infected plants are placed in a humid atmosphere the parasites wander out, and adopt ectoparasitic life in the leaf axils. (3) Leaf to leaf infection experiments in begonias succeed in a humid atmosphere under a bell jar, but fail in the open air.

When chrysanthemum leaves are moistened by heavy dew, adult *Aphelenchi* can be observed (under a binocular microscope) wandering freely over their hairy surface (Molz, 1909).

High humidity assists migration by approximating the conditions of life on the plant surface to those prevailing in the mesophyll spaces. It is not, however, essential to migration (*vide* p. 175, and since *Aphelenchus* disease prevails in the open air).

Sorauer (1902) maintains that *Aphelenchi* cannot invade healthy plants, that a disease of the vascular bundles is produced in pot plants grown at a temperature above the optimum, and that *Aphelenchi* enter such diseased plants only. Marcinowski (1908), on the other hand, infected healthy begonias in a natural manner.

Aphelenchi are attracted by cell sap. I found that they collect in considerable numbers in plant wounds. Molz's theory of entry through wounds is doubtless true in a limited sense; the injuries to which he refers are caused by insect parasites, and by the eruption of axillary buds.

Certain races of many species of flowering plants are immune to the parasite (see *litt. passim* and Molz, 1909).

X. METHODS OF COMBATING THE DISEASE.

Chemical treatment is of use only in such plants as Everlasting (Mangin, 1895), if the blotching appears after the harvest, when the flowers are stored in warehouses. Exposure to a dry atmosphere saturated with carbon bisulphide for 24–48 hours, kills the parasites without affecting the appearance of the plants. In no other plants has chemical treatment of any kind been found of avail, since chemicals of sufficient strength damage the appearance of the plant.

Treatment by heat. Marcinowski (1910)—immersion of the plant in water at 50–52° C. for five minutes, kills the parasite but not the plant.

Prophylaxis based on the habitat of the parasite in the surface of the soil. Earth for filling pots or boxes should be dug from a pit, surface earth being rejected. For open air culture deep ploughing of infected fields may prove useful. All infected leaves and plants should be burned. If it is necessary to use infected stock plants, they should be treated by the hot-water method, or by burying to a depth of at least three inches—after daily watering for at least a week, the surface earth should be removed, after which it is unlikely that any *Aphelenchi* will remain on the plant.

XI. TECHNIQUE.

Fixatives. Carnoy's chloroform-acetic-alcohol mixture, Schaudinn's or Bouin's fluid, and boiling 70 per cent. alcohol are satisfactory; the first-named gives the most clear definition.

Stains for mounting *in toto* haemalum differentiated with acid 70 per cent. alcohol, following Carnoy or Schaudinn is best; after Bouin, picrocarmine gives the best stain.

Mounting. Looss' alcohol-glycerine evaporation method, followed by glycerine jelly for unstained specimens. For stained specimens transfer to xylol, and add canada balsam very gradually on several successive days.

Embedding in paraffin. The tissue should be transferred to chloroform, paraffin (60° C. M.P. for vegetable tissues) added to saturation, the chloroform slowly evaporated in an incubator for 24 hours. Transfer to two baths of melted paraffin for 5 and 10 minutes respectively.

Staining sections. Thionin and eosin give the best results.

SUMMARY.

There are three species of true parasites in the genus *Aphelenchus*, viz.: *A. fragariae* R.B., 1891, *A. olesistus* R.B., 1893, and *A. phyllophagus* n.sp. *A. ormerodis* R.B., 1891, is a doubtful species, possibly a young form of *A. fragariae* (Marcinowski, 1908).

A. fragariae causes the disease strawberry bunch; *A. olesistus* and *phyllophagus* cause leaf disease in flowering plants and ferns. The diseases are widely distributed and of considerable economic importance.

From the study of the anatomy of the three species certain points of general importance emerge: (1) The salivary glands of *Aphelenchus* represent the oesophageal glands of other nematode genera, which have separated from the body of the oesophagus. This separation causes the reduction in size of the posterior portion of the oesophagus, and its lack of clear demarcation from the intestine. (2) The excretory organ of *Aphelenchus*, and of some other nematode genera, originates from the left lateral line. (3) The vaginae are ectodermal organs distinct in origin from the gonads proper.

The life cycle of the parasitic *Aphelenchi* is divided into three definite stages: (A) That of residence in the definitive habitat, which for *A. fragariae* is in the stem and leaves of the strawberry plant, for *A. olesistus* and *phyllophagus* in the mesophyll spaces of the leaves of many plants. Nutrition and reproduction are most actively carried on in this situation, several or many generations succeeding each other, and here the pathological effects are manifest. (B) The resting stage in the soil. The worms reach the soil in fallen leaves, and the adults survive in a partially dried, dormant condition on the surface of the soil for prolonged periods. (C) The stage of immigration into the host plant. When revived by moisture the *Aphelenchi* may be attracted by a suitable plant, and wanders on to it; they may live as ectoparasites in the leaf axils, breeding in this situation, the larvae migrating to the definitive habitat; or they may enter the definitive habitat direct. They do not traverse the tissues of the stem during immigration. *A. olesistus* and *phyllophagus* enter the mesophyll spaces through the stomata.

It should be noted that this life cycle is that of the race, not of the individual. It comprises many generations, and in this respect resembles that of *Tylenchus dipsaci* and differs from that of *T. tritici*, which includes one generation only.

In the life of the individual *Aphelenchus*, embryonic development occupies not more than five days, embryonic and larval development not more than ten, and a complete generation not more than fourteen days.

High atmospheric humidity assists the migration of the worms, previous disease or injury of the host plant may do so. Some races of various species of plants are immune.

Treatment of the affected plants by chemical methods is of limited applicability. Treatment by immersion in water at 50–52° C. for five minutes is recommended by Marcinowski (1908). Prophylaxis should be based on burning of infected plants and leaves, and on the avoidance of infected surface soil in the filling of pots and boxes.

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A, anus; *AF*, *Aphelenchus fragariae*; *AP*, *A. phyllophagus*; *CA*. anterior section of oesophageal collar; *CN*, collar nucleus; *CP*, posterior limit of collar; *D*, dorsal surface; *E*, excretory pore; *GC*, guard cell of stoma; *H*, hair; *I*, intestine; *IL*, intestinal lumen; *LL*, lateral line; *N*, nerve ring; *O*, ovary; *OA*, anterior oesophagus; *OB*, oesophageal bulb; *OE*, endothelium of gonad tube; *OG*, oesophageal gland; *OI*, junction of oesophagus and intestine; *OP*, posterior oesophagus; *OV*, ovum; *P*, papilla; *R*, renette cell; *RE*, rectum; *S*, spear; *SCY*, spermatocyte; *SD*, salivary duct; *SG*, salivary gland; *SGO*, spermatogonium; *SO*, opening of salivary duct into bulb; *SP*, spicule; *SPH*, sphincter; *ST*, spermatid; *STO*, aperture of stoma; *SZ*, spermatozoon; *U*, uterus; *V*, vulva; *VB*, vascular bundle; *VA*, anterior vagina; *VP*, posterior vagina; *VS*, ventral surface.

SUPPLEMENTARY ACCOUNT OF THE DIPTEROUS LARVAE FEEDING UPON MOLLUSCS.

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In my previous paper (*Parasitology*, xi. 1919) on the life history of a Calliphorine fly, *Melinda cognata* Meig., the larvae of which live as parasites in a snail (*Helicella virgata*), I gave a complete account of all the Dipterous larvae feeding upon molluscs.

Since the publication of my paper several new records dealing with this subject have been published or communicated to me by letters. As some of this information is yet unpublished while the rest is scattered in different journals it may be useful to bring together these records and thus to complete the list of observations dealing with the Dipterous fauna of molluscs.

1. **Melinda cognata** Meigen.

Soon after publication of my paper on the life history of this fly, Sir Arthur Shipley wrote a popular account of it, which appeared in *Country Life*, January 3rd, 1920, pp. 14–15. In reply to this article he received from E. Adrian Woodruffe-Peacock a very interesting letter which he forwarded to me. This correspondent writes that he has often observed *Melinda cognata* depositing its eggs on *Helicella virgata*. He has often met with the larvae of this fly and he believes that the huge mortality of *Helicella* observed by him in 1917 was undoubtedly due to them.

2. **Sarcophaga filia** Pandellé.

An interesting account of the life history of this fly has been recently given by J. Rostand (1920). This author found in July of 1916 and 1917 at Camb (Basse-Pyrenées, France) a large number of a small snail (*Helix*) harbouring Dipterous larvae. The adult flies bred from these larvae were identified by Dr Villeneuve as *Sarcophaga filia* (Rond.) Pandellé. According to Rostand the larvae are real parasites since he found them in their young stages attacking almost healthy snails. He compares this case of parasitic life to that of *Melinda cognata*. The larva of *S. filia* completely devours its host and is transformed into the pupa inside the emptied shell of the snail. 15 days later the adult fly emerges. He thinks that there is only one generation *per annum*.

The larvae of *S. filia* are often parasitised by Hymenoptera belonging to the families of Braconidae and Cynipidae.

3. **Sarcophaga melanura** Meigen.

M. E. Séguy informs me that he has observed the females of this fly depositing their larvae upon the slug *Arion fuscus*. In spite of the violent contractions of the slug and the abundant production of mucus the larvae make their way into the body of the host which is soon killed and liquefied.

4. **Engyzops pecchiolii** Rond.

A recent paper of W. R. Thompson (1921) contains much interesting information concerning this fly. He dissected a female of *E. peechiolii* captured in the south of France (Gers) and found its internal genital organs well developed and the uterus filled with completely formed larvae ready to hatch from their eggs. The study of these first stage larvae revealed their close resemblance to the first stage larva of *Melinda gentilis*, which, as we have previously shown (1919, p. 442), lives as a parasite in *Helicella virgata*. The genus *Engyzops* was placed by previous authors among the sarcophagid flies, but according to Thompson the structure of the internal genital organs and the first stage larva show that this insect has more affinity with the Calliphorine genus *Melinda* than with the Sarcophagids.

The life history of *E. pecchiolii* is not yet known, but the resemblance of its first stage larva to that of *Melinda* suggests, according to Thompson, the possibility that in its early stages it also lives on snails.

5. **Lucilia dux and Pycnosoma.**

Under the title "Mortality among snails and appearance of Blue-bottle Flies," we find in *Nature* (1919, CIV. pp. 412–413) a very interesting letter by N. Annandale of the Indian Museum, Calcutta. The following are a few extracts from this letter:

"The residential parts of Calcutta are remarkably free, as a rule, from both house-flies (*Musca* spp.) and blue-bottles. This is doubtless due to the excellence of the municipal sanitary arrangements, for at Sibpur, a few miles away, blue-bottles (*Pycnosoma* or *Lucilia dux*) are not only extremely troublesome in the houses, but are also probably connected with frequent epidemics of enteric, unknown in the better parts of Calcutta. For some years past I have been able to trace the flies to their breeding-ground. This has always been the dead bodies of the snail *Achatina fulica*, the largest land mollusc in Bengal...." "Fortunately it is largely a feeder on decaying vegetable and animal matter, and therefore does little harm to crops or gardens, and has even its value as a scavenger. Since, however, I found the maggots in the dead snails, I have noticed that the appearance of blue-bottles in this part of Calcutta invariably coincides with a heavy mortality in the mollusc, which appears to be subject occasionally to some kind of fatal epidemic and also

perishes in large numbers after egg-laying at the beginning of the rainy season and during dry spells in and at the end of that season.... "

6. **Sciomyza dubia** Fln.

In his report on land and fresh water Mollusca observed in Hertfordshire, C. Oldham writes (1912, p. 288): "Among some Mollusca collected in February on a hedge bank at Tring, I found dead shells of *Vitrea rogersi*, *V. nitidula*, *V. cellaria* and *Pyramidula rotundata*, each of which had in its last whorl a small reddish pupa. From these several specimens a dipteron, *Sciomyza dubia*, hatched out in April. Almost nothing is known of the life-history of this fly, and I cannot say whether the larva is parasitic on the living Mollusc, kills and subsequently devours it, eats the body of the snail which has died from some independent cause, or merely chooses the empty shell as a convenient place in which to pupate."

In a letter to me, dated 26. xii. 1919, C. Oldham mentions that in the autumn he often finds on the chalk downs near Berkhamstead, snails (*Helicella* and others) which harbour fly larvae of an undetermined species; these larvae are sometimes seen free in the collecting tubes containing the snails.

7. **Phoridae.**

Several species of Phorids which usually inhabit the dead snails have been recently carefully studied by Lundbeck (xii. 1919) and as a result of his study the following changes have to be introduced in my previous list of these inhabitants of snails: the *Phora* sp. No. 2, the pupae of which I have found in *Helix nemoralis* collected in the Bernese Oberland, Switzerland (1911, p. 60, and 1919, p. 450), have been identified by Lundbeck as *Phora* or *Paraspiniphora notata* Zett.

Phora bergenstammi Mik. The Phorid, the complete life history of which I described under this name (1911, pp. 31–56, and 1919, p. 449), was proved by Lundbeck to be *Phora domestica* Wood (1906).

Phora domestica Wood was considered by Malloch (1910) to be synonymous with *Ph. bergenstammi* Mik., and this was accepted in 1912 by Wood himself. Lundbeck has proved, however, that these two species, although closely allied, differ in several respects.

Phora bergenstammi Mik. has "4 dorsocentral bristles in both sexes, and the male palpi almost nude"; "the costa (in both sexes) reaches beyond the middle of the wing, the first costal division is at most double as long as the second, and the fourth vein is rather strongly curved at the base."

Phora domestica Wood has "4 dorsocentral bristles in the female and 2 in the male and the male palpi with normal armature..."; "the costa in both sexes does not reach quite to the middle of the wing, the first costal division is more than double as long as the second and the fourth vein is less curved at the base; further, *domestica* seems always to have pale hind margins to the abdominal segments not present in *bergenstammi*."

As to the puparia of these two species they also differ: "By examination of the puparia of *domestica* sent me from Dr Schmitz it was proved, that these puparia are identical with the puparium described by Keilin (*Bull. Sci. France et Belgique* (7), 45, 1911, 47) as *bergenstammi*, so that the species treated by Keilin under that name is in reality *domestica*. An easily observable difference between the two puparia is, that while that of *domestica* has at the lateral margin of each segment two papillae, a small and a larger, with bristles on the apex, there are in *bergenstammi* on each segment three such papillae, a small, a medium sized and a large (Fig. 2)."

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NOTE: Professor A. E. Boycott, F.R.S., has kindly called my attention to the following few misprints which he found in my previous paper (1919):

pp. 433 and 435, instead of *Hygromyia* read *Hygromia*.
p. 434, para. 2, and in References, instead of *Steep* read *Step*.
p. 435, para. 1, instead of *rufiscence* read *rufescens*.
p. 442, para. 5, and p. 448, para. 2, instead of *cantioniformis* read *cantianiformis*.

He informs me also that "*H. virgata* is hardly 'very common' in England and Wales. In many parts of the west and north it is quite a rare species, especially inland."

ON THE NEPHROCYTES OF *PEDICULUS HUMANUS*.

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and D. KEILIN, Sc.D.,
Beit Memorial Research Fellow.

(*From the Quick Laboratory, University of Cambridge.*)

(With 5 Text-figures.)

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Introduction.

Dissections of the thorax of *Pediculus humanus* ♂, ♀, or larva, demonstrate the existence of two groups of special cells lying suspended on either side of the oesophagus between the reniform salivary glands and the forwardly protruding intestinal caeca. These cells have already been observed by entomologists who have studied the anatomy of the louse; Patton and Cragg (1913, p. 559, Pl. LXX, figs. 1 and 2) were the first to see and figure them. These authors refer to them as follows:

"Embedded among the fat body, immediately anterior to the kidney-shaped glands, there is on each side a small collection of round cells, readily distinguished from the fat body by their more glistening appearance. These are constant in position, and are always found, in sections and dissections, surrounding the oesophagus, though not attached to it in any way. They have no capsule, but appear to be connected with one-another by short and pointed processes. The protoplasm of these cells is vacuolated, and contains many fine granules. One, or sometimes two, nuclei are present, and appear as clear vesicles containing a darkly staining mass of chromatin. No duct has been distinguished with certainty, though in some dissections a fine

filament which may perhaps be a duct has been noted passing upward with the salivary duct."

These cells were also observed by Müller (1915, p. 15), who described them in a few lines in his chapter on the fat body. The only interesting statement he makes is that these cells owing to their size, shape and occurrence in groups, recall the pericardial cells of other insects but differ therefrom, however, in respect to their position.

Sikora (1916, pp. 57–58) subsequently gave a good description, calling them "large celled glands" ("Grosszellige Drüsen"). She studied them in sections and found that there were 46–56 cells ("Drüsen") on each side of the oesophagus, although she stated that the number may be much smaller because it was possible that she counted some of the cells twice over. She found that the cells are binucleate and frequently show cleft-like spaces ("spaltförmige Safträume (?)") between their nuclei. The cells are oval and often prolonged into a filament; other very fine filaments connect them to each other and serve to attach them to the surrounding organs. Sikora found no efferent canal connected with the cells and supposed that their function consists in taking up certain substances from the perivisceral fluid, either storing, transforming, or eliminating them again into the perivisceral fluid. She compares these cells to the binucleate cells found by her in the vicinity of the heart in transverse sections of *Haematopinus eurysternus*.

The foregoing paragraphs afford a summary of all that has been published hitherto regarding the structure of these cells.

In the course of our studies on *Pediculus*, we have been able to confirm the statements of the above-cited authors regarding the structure of the cells in question. We have, however, found cells of a similar structure in other parts of the body of the louse and have succeeded in determining the hitherto unknown function of all these elements.

For reasons to be given presently, we shall henceforth refer to these binucleate (at times trinucleate) elements as *Nephrocytes*. The elements referred to by the above-cited authors will be termed by us *peri-oesophageal nephrocytes* (ventral, in clusters), the others will be termed *disseminated nephrocytes* (dorsal).

Peri-oesophageal Nephrocytes in Pediculus.

The number of nephrocytes belonging to this category is certainly inferior to what Sikora supposed, for we have been unable to count more than 16 to 20 on each side of the oesophagus. In sections the nephrocytes appear as large binucleate or at times trinucleate cells possessing very granular protoplasm (Fig. 1). The cleft-like spaces of Sikora appear to be artefacts due to fixation, since they are not visible in the living tissue. The nuclei are vesicular, and, like the nuclei in the other tissues of the louse, they are very poor in chromatin. The chromatin is reduced to a few granules, the rest of the nucleus being clear.

The nephrocytes, when examined alive (Fig. 2), are seen to contain numerous droplets of variable size, which are of a greenish colour. These droplets are larger and more numerous in fasting lice. The size of the droplets at times exceeds that of the nucleus, which may consequently be obscured. The droplets are not dissolved and not decolourized by 80 per cent. alcohol or by weak acetic acid.

The nephrocytes are linked to each other by very fine connective filaments and *they have no duct or the like which communicates outwardly or with the alimentary canal.*

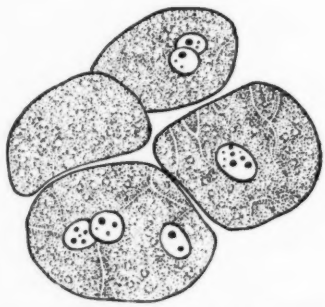


Fig. 1. Peri-oesophageal nephrocytes of *P. humanus* in section (fixed in Carnoy, stained by iron-haematoxylin).

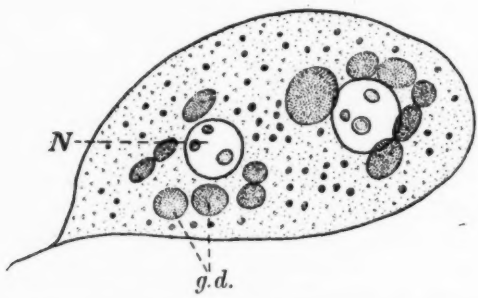


Fig. 2. A living nephrocyte of *P. humanus* slightly compressed, showing two nuclei, *N*, and green droplets, *g.d.*

Disseminated Nephrocytes in Pediculus.

To examine these structures, the living insect should be opened and the fat body removed and spread in a drop of normal salt solution. The specimen is then covered lightly with a cover-glass and examined microscopically. The large polynuclear cells of the fat body, filled with fat droplets, are seen to be connected by much smaller cells. These cells are binucleate and in respect to their protoplasmic structure and inclusions conform to the above described peri-oesophageal nephrocytes, but for their disposition and shape (Fig. 3).

They are more elongated and are more or less fused in a chain-like manner, the terminal nephrocyte being either fused with the fat body or connected therewith by means of a long filament.

The Physiological Function of the Nephrocytes.

The cytological structure of the nephrocytes we have considered above, strongly resembles that of the pericardial cells of other insects as already pointed out by Müller. It is therefore natural to ask if the function of these cells is not similar to that of the pericardial cells. With a view to replying to this question it is necessary to dwell upon what is known regarding the physiological function of pericardial cells.

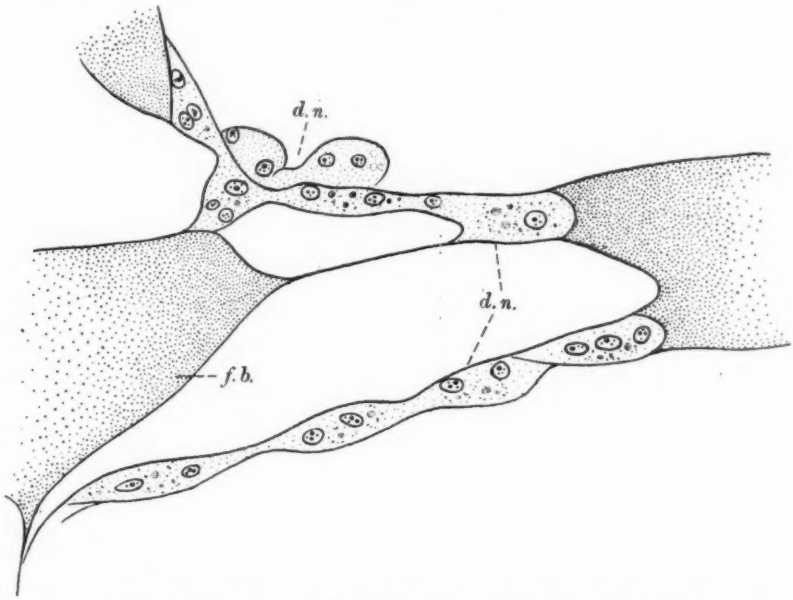


Fig. 3. Disseminated nephrocytes of *P. humanus*, *d.n.*; connected with the fat body, *f.b.*

Kowalevsky (1886) was the first to show that in Dipterous larvae, fed upon artificially coloured food, the pericardial cells become stained more or less intensely. He also showed that the colouring substances he employed, namely cochineal powder and silver salts, become deposited in the protoplasm of the pericardial cells without, however, staining their nuclei.

In 1889, Kowalevsky, moreover, showed that these cells are capable of eliminating certain substances introduced into the body either (*a*) directly by injection into the body cavity, or (*b*) indirectly through the alimentary canal, and, since the cells do not possess an excretory duct, they store throughout their life the substances which they take up.

In his injection experiments Kowalevsky used various substances like ammonia-carmine, indigo-carmine, methylene blue, alizarine, and finally blue litmus on the advice of Metchnikoff. The employment of litmus was of special value since it showed that the pericardial cells possess an acid reaction, for, when the animal is injected with blue litmus the cells turn the absorbed litmus red. Kowalevsky therefore concluded that the pericardial cells constitute excretory accumulatory organs having an acid reaction. It may be noted here that the Malphigian tubes of insects possess a distinctly alkaline reaction.

In 1892, Kowalevsky, whilst confirming his previous observations, found that the pericardial cells are incapable of taking up solid particles, these being taken up by lymphocytes or leucocytes only.

The chief results of Kowalevsky were confirmed by Cuénot (1896) and Metalnikoff (1896). Subsequently Bruntz (1903) in his studies upon excretion in Arthropods, wherein he injected ammonia-carmine and indigo-carmine, was able to show that a great variation exists in the form, grouping and distribution of the excretory cells to which he applied the name "*nephrocytes*." He found these frequently situated at a distance from the heart and lying scattered between the lobes of the fat body. Finally, Keilin (1917) has described the nephrocytes in several species of dipterous larvae, and observed cases (*Lonchae vaginalis*) where the nephrocytes under natural conditions were coloured, according to the age of the larva, either light brown or black by the accumulation within them of excretory substances.

To determine the excretory function of the nephrocytes in *Pediculus*, it was necessary to employ one of two methods that have been used in similar studies upon other insects, the two methods being (1) to feed the insects with coloured fluid, or (2) to inject such coloured matter into their body cavity. Owing to lice being solely blood-sucking insects, we were obliged to carry out our experiments by the second method only. After a few preliminary trials we succeeded perfectly in obtaining satisfactory results, and, since it may prove useful to other investigators, we shall describe our method of performing such experiments.

Method of performing Intra-coelomic Injection upon Pediculus.

The first step is to prepare fine glass pipettes from tubes measuring 1 mm. in diameter and 7–8 cm. in length. One end of the tube is drawn out to a fine but rigid point which is broken off with a forceps under the binocular microscope so that a fine lumen can be discerned.

The best results are obtained with lice maintained in the thermostat at 30° C. for about 16 hours after a meal, replete lice being unsuitable for injection. Holding the louse between the forefinger and thumb of the left hand, the point of the pipette, charged with a small amount of injecting fluid and held in the right hand, is driven into the side of the insect's abdomen, gentle pressure only being exerted. As soon as the point has traversed the external

integument, the insect should be released by the fingers, whilst it remains impaled on the pipette point. The operator now places the free end of the pipette between his lips and blows some of the fluid it contains into the insect's body cavity. As soon as this is accomplished the louse is detached from the pipette by sliding a needle along the glass. The insect, after resting on cloth at room temperature for an hour, is fed and afterwards returned to the thermostat. After 20–24 hours, if the louse has been injected with ammonia-carmine, the nephrocytes of the living specimen are found to be coloured red when viewed by transparency.

We would remark that before reaching conclusions regarding experiments of this character it is necessary to confine one's attention solely to the living insects because more or less generalized coloration may take place in dead or dying lice.

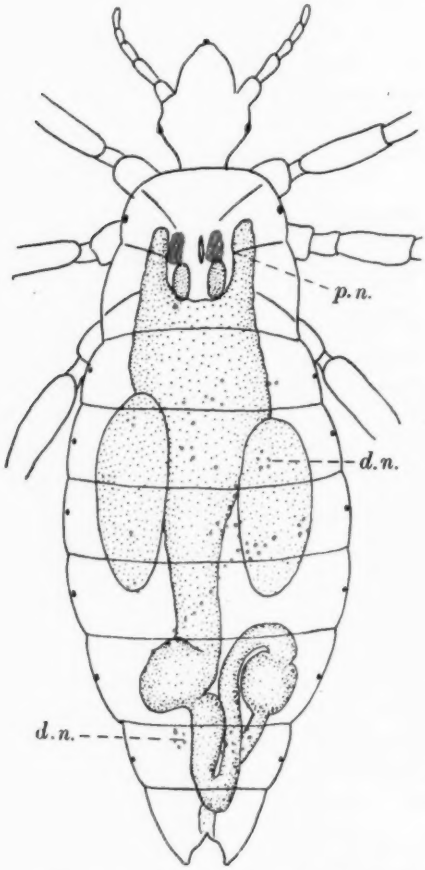
Results of Intra-coelomic Injections of ammonia-carmine in Pediculus.

Of 22 experimental injections of the foregoing description carried out by us on *Pediculus humanus*, 14 gave good results. The failures were due either to the lice being injured through laceration of the gut or through precipitation of the injected carmine having occurred. The successfully treated lice were killed for purposes of examination 3–5 days after injection. One specimen (♀) injected on 4. vi. 1918, which showed coloured nephrocytes after 24 hours, survived until 21. vi, when she was killed. In other words she lived for 16 days, and laid 62 eggs during this period, her nephrocytes remaining coloured throughout. It is evident therefore that the technique when successful does not involve material injury to the louse.

When the lice are examined 24 hours after injection no traces of ammonia-carmine are visible in the perivisceral fluid, on the other hand all the nephrocytes are found to be coloured red. When the lice are immersed in water and slightly compressed between slide and cover-glass, being viewed dorsally with the binocular microscope, the exact distribution of the nephrocytes can be clearly discerned. As shown in Fig. 4, as a rule, two red peri-oesophageal agglomerations of nephrocytes are visible, at times the masses are fused in the form of an irregular crescent.

Apart from the foregoing, small groups of red cells are seen irregularly distributed beneath the dorsal surface. The distribution of these cells varies in different individuals and in one and the same individual according to its state of repletion, or if the specimen is a female, according to the state of development of the ova. These cells or groups of cells, when dissections are made, are seen to be united to the fat body as shown in Fig. 5, and they correspond in every way to the cells we have already described as dorsal or disseminated nephrocytes. When examined under a high power, they exhibit the two characteristic nuclei which are hidden in the illustration through the accumulation of carmine granules within the cells.

In two cases the nephrocytes were so charged with granules of carmine that they began to break up thereby liberating carmine granules which were promptly taken up by the leucocytes in the perivisceral cavity. Soon after-



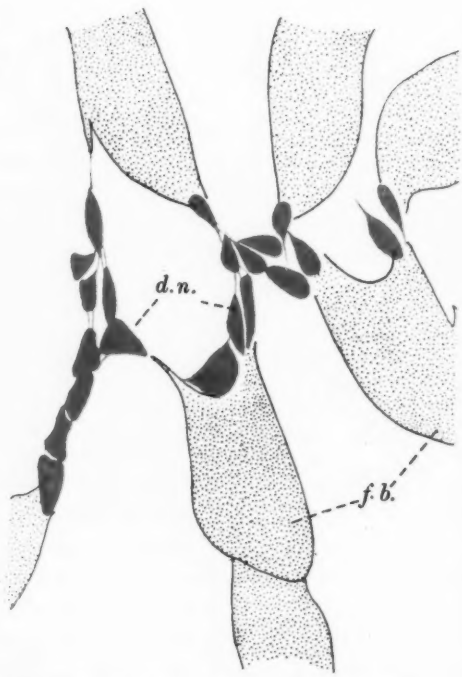


Fig. 4. *Pediculus humanus* ♀, 24 hours after being injected with ammonia-carmine, showing the nephrocytes filled with carmine: *d.n.*, disseminated dorsal nephrocytes; *p.n.*, peri-oesophageal or ventral nephrocytes.

Fig. 5. Dorsal or disseminated nephrocytes linked with the fat body cells, extracted from *P. humanus* after injection with ammonia-carmine: *d.n.*, dorsal nephrocytes; *f.b.*, fat body.

wards, in all parts of the body, even in the head and legs, were found isolated or aggregated leucocytes charged with carmine granules, the appearance being identical with that following the injection of a suspension of powdered carmine into the body cavity of an insect.

Nephrocytes of Mallophaga.

Essentially similar to the peri-oesophageal nephrocytes of *Pediculus* are certain groups of cells described by various authors in Mallophaga. Thus Kramer (1869, p. 455) working with *Lipeurus jejunus*, found, connected with the smaller pair of salivary glands, a peculiar complex of about 14 large cells grouped in two rows, the cells "mostly containing two or four nuclei." These cells showed no efferent duct, but, "because of their constant seat of attachment at the end of the gland opposite to where its duct issues," they were regarded by Kramer as necessarily bearing some relation to the salivary secretion.

Grosse (1885, p. 549) found similar cells to those described by Kramer in *Nirmus*, *Trichodectes*, *Lipeurus versicolor* and *L. heterographus*. The cells were not, however, confined to the crop region, but also occurred in other parts of the body in groups of 2–6–8 upon the fat body. Grosse argued that since the cells are so generally distributed in the insect's body their salivary function, as supposed by Kramer, is doubtful.

Snodgrass (1899, pp. 167–8) on the other hand, figured and supposed that he discerned an efferent duct leading from similar cells in *Trichodectes geomydis*, the duct opening at the junction of the crop and oesophagus. In *Eurymetopus*, *Docophorus* and *Gonoides*, the cells are more numerous than in *Trichodectes* and their "ducts" are difficult to see. According to Snodgrass each "cell possesses two nuclear-like bodies (one of these may be a hollow space into which the duct opens, such spaces being present in salivary cells of insects), and they are all closely pressed together so that they assume polygonal shapes." The observations of Snodgrass are not in accord with those of Kramer and Grosse, and they appear very doubtful because the nephrocytes are usually tangled up with tracheae, nerves, muscle fibres and real salivary ducts, any of which Snodgrass may have taken for a "duct" leading from the nephrocytes.

Nephrocytes of Pediculus *and Diptera compared.*

There remains to be considered the distribution of the nephrocytes observed in dipterous larvae since they are comparable with those in *Pediculus*.

According to Weismann, Kowalevsky, Bruntz and Keilin, the nephrocytes in dipterous larvae are distributed in two distinct groups: (*a*) One group occurs in the form of two parallel dorsal cell-chains applied to the sides of the heart; these are the pericardial cells in the strict sense. (*b*) The second group of nephrocytes forms a festoon-like chain ("girlandenförmiger Zellenstrang" of Weismann) that is always found suspended ventrally between the two salivary glands (see Keilin, 1917, pl. XII, fig. 46 n.v.). It is highly probable that the two groups of nephrocytes in dipterous larvae correspond respectively to the disseminated nephrocytes (dorsal) and peri-oesophageal nephrocytes of *Pediculus*, especially since according to Sikora (p. 58) in *Haematopinus eurysternus* the dorsal nephrocytes occupy a pericardial position.

Fulmek (1909, pp. 56–58, pls. I, II), moreover, has shown that in Mallophaga the pericardial cells are closely aggregated at the sides of the heart.

SUMMARY.

In *Pediculus humanus* are found two groups of excretory-accumulatory cells known as nephrocytes.

The one group, the peri-oesophageal, lies ventrally and consists of large cells aggregated usually in two masses about the oesophagus anterior to the reniform salivary glands.

The second group lies dorsally and consists of disseminated cell aggregates linked with the fat body.

The typical nephrocyte is a binucleate cell with granular protoplasm containing greenish droplets of varying size. The excretory function of the nephrocytes is demonstrated by intra-coelomic injection of ammonia-carmine. The latter, 24 hours after injection, is taken up by the nephrocytes which become red and filled with the carmine granules. These granules remain in the protoplasm of the nephrocytes throughout the life of the insect.

Similar cells exist in Mallophaga and have been wrongly described by some authors as salivary glands.

The two groups of nephrocytes, described by us in *Pediculus*, occur in other insects, but the dorsal group in the latter forms usually two chains of cells (known as pericardial cells) lying on either side of the heart.

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Owing to the greatly increased cost of publication authors are earnestly requested to present their papers in as *concise* a form as possible. Wordiness too frequently causes delay, trouble and unwarranted expense. Unnecessary details relating to experiments should be omitted and *tabular matter* especially should be curtailed to a minimum because of its high cost.

Manuscripts.

MSS. should be *type-written* or at least written in a legible hand on one side of each sheet of paper. The pages of the MS. should be numbered. They should be *carefully prepared for press* so as to lessen the cost of corrections. It is an all too frequent burden upon the Editor to have to correct the grammar, spelling and punctuation of inconsiderate writers. Sufficient space should be allowed between the lines for alterations if required. The divisions in the text should be indicated by appropriate *headings*, corresponding in wording to those enumerated in the table of *Contents* that in most cases should follow upon the title of the paper. A multitude of small paragraphs is not desirable.

Care should be taken that *the title* of the paper shall clearly indicate its contents. Where scientific names of animals or plants occur in the title, provided such names are not commonly known, authors should state to what Order or to what Order and Family they belong, this being added in parenthesis as indicated in the following examples: *Melinda cognata* Meigen (Diptera, Calliphorinae), *Maupasella nova* Cépède (Ciliata, Astoma), *Cladorchis subtrietrus* Rudolphi (Trematoda). Authors who observe this rule will greatly facilitate the work of readers, indexers and others who deal with the classification of scientific literature.

If any part of the MS. is to be printed in small type kindly indicate by adding a line at the side of copy.

Tables, *Legends* and *References* should be written on separate sheets from the rest of the manuscript since they are printed by means of a different type. Authors should be sparing in the use of footnotes. Tables, legends, etc., should be numbered and the place where they are to be inserted in the text should be indicated by corresponding numbers in the margin of the MS.; authors should not refer to the "foregoing" or the "following" table since the position of the table or reference on the printed page does not necessarily agree with that on the manuscript.

Measures and Abbreviations. The metric system of measurements should be used, abbreviations being employed as follows: *cm.* (centimetre), *mm.* millimetre), μ (micron), *kgm.* (kilogramme), *gm.* (gramme), *cgm.* (centigramme),

mgm. (milligramme), *l.* (litre), *c.cm.* or (as commonly employed by English writers) *c.c.* (cubic centimetre), *c.mm.* (cubic millimetre).

Decimal points should never be placed at the beginning of a number (thus ·5); the form 0·5 should be used because the point may be overlooked by compositors and proofreaders or be broken in printing, thereby leading to regrettable or absurd errors.

Centigrade and Fahrenheit. Where the author has employed the Fahrenheit scale, the equivalent Centigrade measurement should be stated for the convenience of the majority of readers who employ the Centigrade scale. The Fahrenheit measurement should be given the second place in a bracket, thus: 100° C. (212° F.).

Whenever it is possible, authors are requested to end their papers with a brief *Summary* of the conclusions to which they have arrived. This is essential, especially with regard to lengthy papers.

All references should be given in full, either as footnotes or, particularly if there be many references, arranged alphabetically by authors at the end, the date of the paper being bracketed alongside the author's name. Cite also the volume and the page thus:

HAYWARD, T. E. (1902). On the construction and use of life-tables from a public health point of view. *Journal of Hygiene*, II. 1–42.

Authors will find it much easier in the end if they will cite the author by giving (in the text) the date and page of the paper referred to, thus: "Hayward (1902, p. 6) found, etc." By adopting this method, known as the "Harvard System," a new reference can be slipped into the MS. or proof without completely disturbing the order of references as is the case when papers are referred to in the text by the usual, but antiquated, method of citing the papers in accordance with the numbers to which they correspond in the list of references at the end of the paper. Moreover, the reader can readily be guided to the particular page whence the citation is derived.

The *names of Journals and titles of books should be in italics* (underline in MS.) and written out in full. The Editor will see that these names are abbreviated according to standard methods where necessary. Abbreviations like "B.M.J.," "J.R.A.M.C.," "Q.J.M.S.," "C.R.A.S.," are incomprehensible to many readers and a thorn in the side to Editors. By using Roman numbers for volumes and Arabic numbers for pages "vol." and "p." may be omitted, thus "XII. 23" denotes vol. XII. p. 23, whereby needless type-setting is avoided. Authors should know that the abbreviations "T." in French and "Bd." in German stand for "vol."

In quoting the titles of Journals or books in languages other than English the writer should be particularly careful to see that the words are spelt correctly and that the proper accents, if any, are added to them. Much time is often lost in the endeavour to detect errors of this character in the final proofs, and also in rectifying them in the type.

Scientific names of animals and plants should be italicised in accordance with the rules of nomenclature, only the generic names should begin with Capitals, e.g. *Bacillus diphtheriae, Ascaris lumbricoides, Trypanosoma brucei*, etc.

Diagrams and Figures should always be accompanied by full descriptions or legends.

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Illustrations constitute a most expensive item in publication. Authors should therefore select only those which are essential. Illustrations should never be placed in the text, they should be on separate sheets, the figures being clearly numbered in accordance with the numbered descriptions or legends.

Authors will be expected to contribute to the cost of their plates or to obtain grants covering a reasonable share of the expense entailed.

Authors should realise the great difference there is in the relative cost of various modes of illustration. If the cost of 500 copies of a plate reproduced by Coloured lithography requiring the use of six stones be taken as 100, then the cost of the other processes commonly employed would work out somewhat as follows: Three-colour process, 75; Monochrome lithography, 50; Collotype, 42; Photo-lithography, 27; Halftone, 23; Line block, 11. Naturally the cost is governed in all cases by the amount of detail in the figures and their size when reproduced.

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Where *photographs* are to be reproduced by the half-tone process, a good positive is required, negatives are unsuitable. Photographs should be trimmed down so as to show what is essential only.

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Charts and Curves are frequently drawn to best advantage on *graph paper ruled in pale blue* since, whilst ensuring accuracy, the blue lines do not appear in the photograph, only the blackened lines that are desired remaining.

Proofsheets.

Authors will receive one set of slip proof for correction and return to the Editor. An allowance of ten shillings per sheet of sixteen pages will be made for alterations apart from printer's errors. Authors will be charged for any excess over the amount stated.

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G. H. F. N.